

ELECTROCHEMICALLY ACTIVE LIGAND FOR SEQUENCE-SPECIFIC  
DETECTION OF DOUBLE-STRANDED NUCLEIC ACID MOLECULE

FIELD OF THE INVENTION

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The invention relates to an electrochemically active ferrocene compound that may be used as a ligand for sequence-specific detection of a double-stranded nucleic acid molecule and the like.

10

BACKGROUND ART

"DNA microarray" or "DNA chip" is a collective term that means slide glass or silicon base having DNA sequence 15 fragments aligned in a high density thereon, which is an analytical tool used for gene expression analysis that have been recently developed. Transcription amount of each gene may be detected by using as an index intensity of hybridization of a prepared RNA or DNA sample (target).  
20 Such DNA microarray has now been used for the detection of single nucleotide polymorphism (SNP) as well.

The typical DNA chips that are known to be commercially available are an "Affimetrix-type" one sold by Affimetrix 25 (USA) and a "Stanford-type" one developed by Stanford University (USA). The Affimetrix-type chip is made by synthesis of a DNA probe on silicon base in a high density so that it can fix from several thousands to several tens

of thousands of kinds of probes on one chip. On the other hand, the Stanford-type chip is made by dropping DNA fragments that have been prepared in advance on slide glass. The detection with those chips is usually carried out by 5 image analysis with the use of fluorescent pigments bound to the target.

Recently, a technique has been developed in which the results of hybridization between the probe and target are 10 detected electrochemically instead of fluorescent pigment (electrochemical Array: ECA chip) (Drummond, T.G.; Hill, M.G.; Barton, J.K. Natl. Biotechnol. 203, 21, 1192-1199). Such electrochemical detection is performed with the use of an intercalating agent that is electroconductive and 15 having a property of being intercalated between neighboring base pairs of a double-stranded DNA. There are already known electroconductive intercalating agents such as anthraquinone, naphthquinone, polyphyne and ferrocene. An example of the detection of a gene using ferrocene 20 derivatives was reported(Fan, C.; Plaxco, K.W.; Heeger, A. J. Proc. Natl. Acad. Sci. USA, 2003, 100, 9134-9137). Furthermore, a DNA chip with the use of ferrocenyl naphthalene diimide derivatives as the intercalating agent and various methods using said chip have been proposed 25 (Japanese Patent Application Publications No.2003-300 and No.2003-83968).

On the other hand, hairpin-type

pyrrole-imidazole-polyamide (PIPA) usually forming 6-mer or 8-mer is known as a prototype of synthetic organic molecules that can site-specifically recognize a DNA sequence, and its potentiality for gene regulation has been 5 therefore proposed (Mrksich, M.; Parks, M. E.; Dervan, P.B. J. Am. Chem. Soc. 1994, 116, 7983-7988). Furthermore, many attempts have been reported in which the structure of PIPA was modified in order to alter their functions and properties. For example, some research groups have 10 proposed modification of PIPA with a fluorescent pigment (Rucker, V.C.; Foister, S.; Melander, C.; Dervan, P.B. J. Am. Chem. Soc. 2003, 125, 1195-1202), or an alkylating agent (Wang, Y-D.; Dziegielewski, J.; Wurtz, N.R.; Dzielewska, B.; Dervan, P.B.; Beerman, T. A. Nucleic Acids Res. 2003, 15 31, 1208-1215, et al.), in order to use it as a tool for the detection of a gene and a potential anti-cancer agent.

In addition to the above functional modification, some other research groups tried the modification of the 20 structure of PIPA into other heterocyclic compounds for the purpose of improvement of affinity and sequence-specificity of pyrrole and imidazole for the double-stranded DNA, and some of them reported success of the improvement of sequence-specificity (Foister, S.; Marques, M. A.; Doss, R.M.; Dervan, P.B. Bioorganic Med. 25 Chem. Lett. 2003, 11, 4333-4340, et al.)

[Patent Document 1] Japanese Patent Application

Publications No.2003-300

[Patent Document 2] Japanese Patent Application

Publications No.2003-83968

[Non-Patent Document 1] Fan, C.; Plaxco, K.W.; Heeger,

5 A. J. Proc. Natl. Acad. Sci. USA, 2003, 100, 9134-9137

[Non-Patent Document 2] Mrksich, M.; Parks, M. E.; Dervan,  
P.B. J. Am. Chem. Soc. 1994, 116, 7983-7988

[Non-Patent Document 3] Rucker, V.C.; Foister, S.;  
Melander, C.; Dervan, P.B. J. Am. Chem. Soc. 2003, 125,

10 1195-1202

[Non-Patent Document 4] Wang, Y-D.; Dziegielewski, J.;  
Wurtz, N.R.; Dzielewska, B.; Dervan, P.B.; Beerman, T. A.  
Nucleic Acids Res. 2003, 31, 1208-1215

[Non-Patent Document 5] Foister, S.; Marques, M. A.; Doss,  
15 R.M.; Dervan, P.B. Bioorganic Med. Chem. Lett. 2003, 11,  
4333-4340

#### SUMMARY OF THE INVENTION

Problems to be solved by the invention

20

The intercalating agents that have been conventionally used  
for the electrochemical DNA chip have disadvantages that  
they may bind not only to the double-stranded DNA but also  
single-stranded DNA so as to generate other electrical  
25 signals than that based on the double-stranded DNA, or that  
a noise current generated by free intercalating agents will  
cause a detection noise, which will then increasing  
electrochemical signal/noise ratio (S/N).

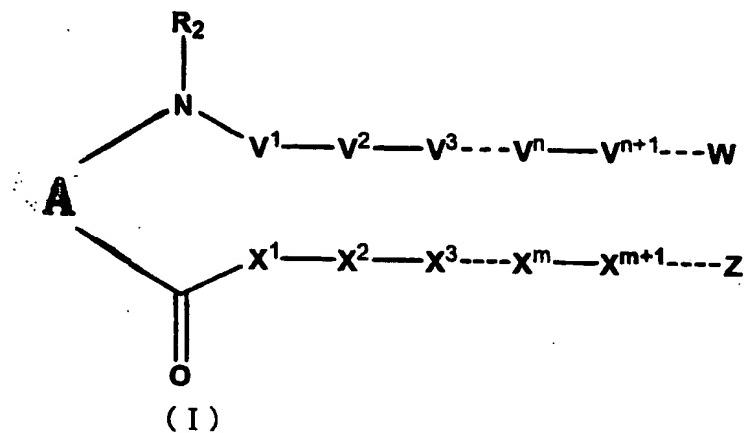
It is well known that an authentic nucleotide may form a considerably stable mismatch base pair such as G-T, A-G and G-G pairs, which may generate false-positive signals  
5 in the above electrochemical detection methods using the conventional intercalating agents.

The present inventors have studied to develop a compound that can specifically recognize the base sequence of  
10 double-stranded nucleic acid molecule by means of modification of a linker region in the pyrrole-imidazole-polyamide structure. Finally they have succeeded in the synthesis of a compound having a ferrocene moiety in the linker region of the  
15 pyrrole-imidazole-polyamide structure, and overcome the above problems.

Means for solving the above problems

20 Thus, the present invention relates as a first aspect to a ferrocene compound represented by the following general formula (I):

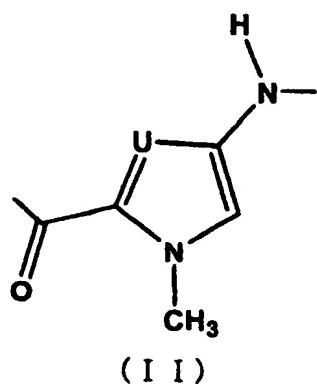
[Chemical Formula 1]



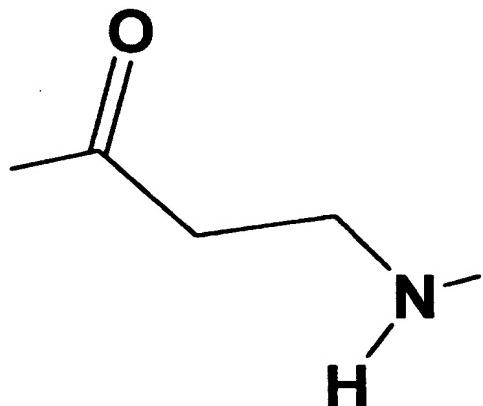
wherein "A" represents a divalent ferrocene-containing  
 5 linker or ferrocene-1,1'-yl,  $R_2$  represents a hydrogen atom  
 or alkyl; "n" and "m" represent any natural numbers; and  
 "V" and "X" represent the following general formula (II)  
 or (II-1):

[Chemical Formula 2]

10



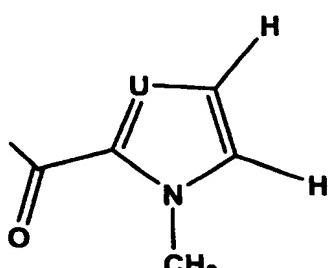
[Chemical Formula 3]



(II-1)

"W" represents the following general formula (III):

5 [Chemical Formula 4]

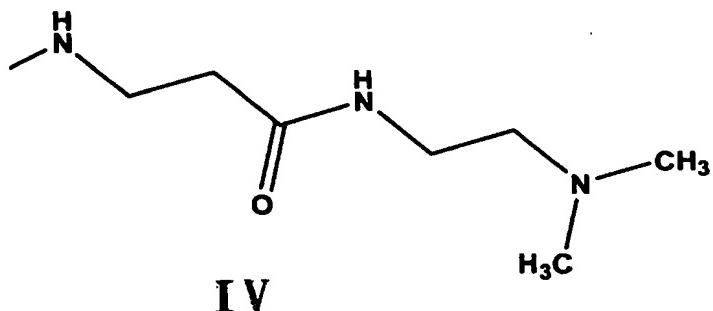


III

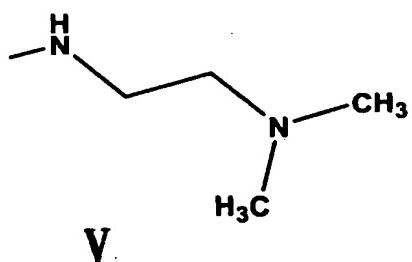
wherein "U" in the general formulae (II) and (III)  
represents a nitrogen atom, methine or hydroxymethylene; and

10 "Z" represents the following general formulae (IV) or (V):

[Chemical Formula 5]



[Chemical Formula 6]



5

and both ends of each of V<sup>n</sup> and X<sup>m</sup> in the general formula (I) form a (-CO-NH-) bond except that a bond on the side of the ferrocene-containing linker or ferrocene-1,1'-yl of V1 is (-CO-NR<sub>2</sub>-).

10

The present compound represented by the general formula (I) consists of the divalent ferrocene-containing linker and two pyrrole-imidazole-polyamide (PIPA) regions bound to the linker.

15

The ferrocene-containing linker may therefore take any structure as long as it contains a ferrocene group and is compound (or atom group) showing the function mentioned above.

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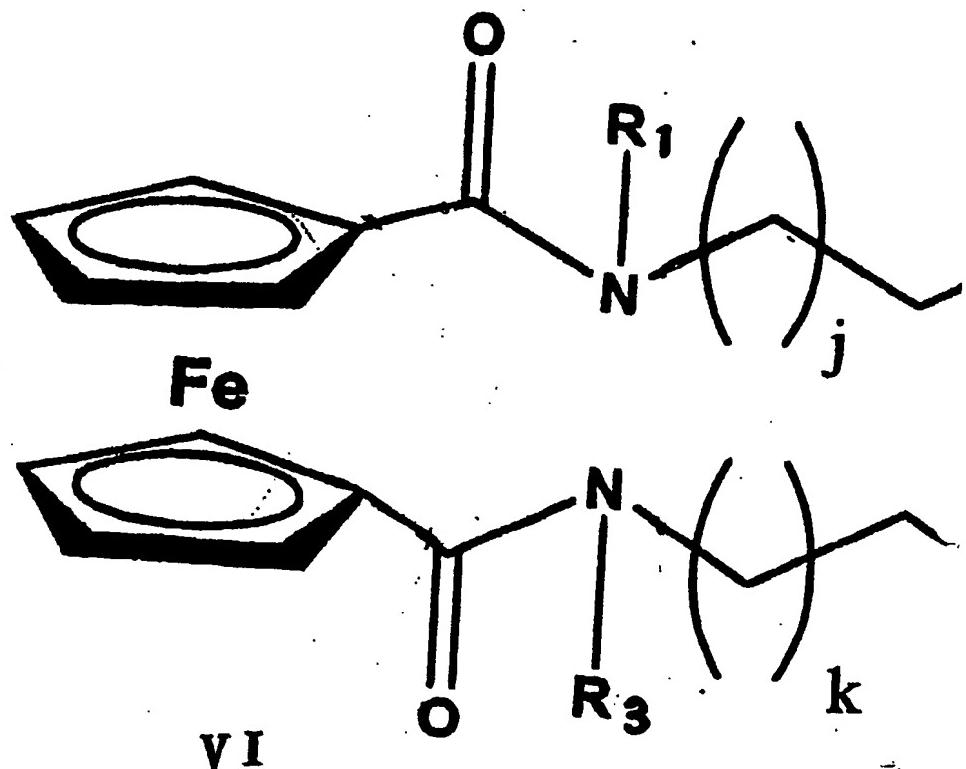
The numbers "n" and "m" are natural numbers, preferably in the range of 3 - 20, more preferably of 3 - 10. Furthermore, it is preferable that the number of "n" is smaller by one than that of "m" so that the total numbers of imidazole 10 and pyrrole derivatives in the atom groups bound to the both sides of the ferrocene-containing linker will be finally the same to each other.

Preferable examples of the ferrocene-containing linker (A) 15 include the compounds represented by following general formulae (VI), (VII) and (X) :

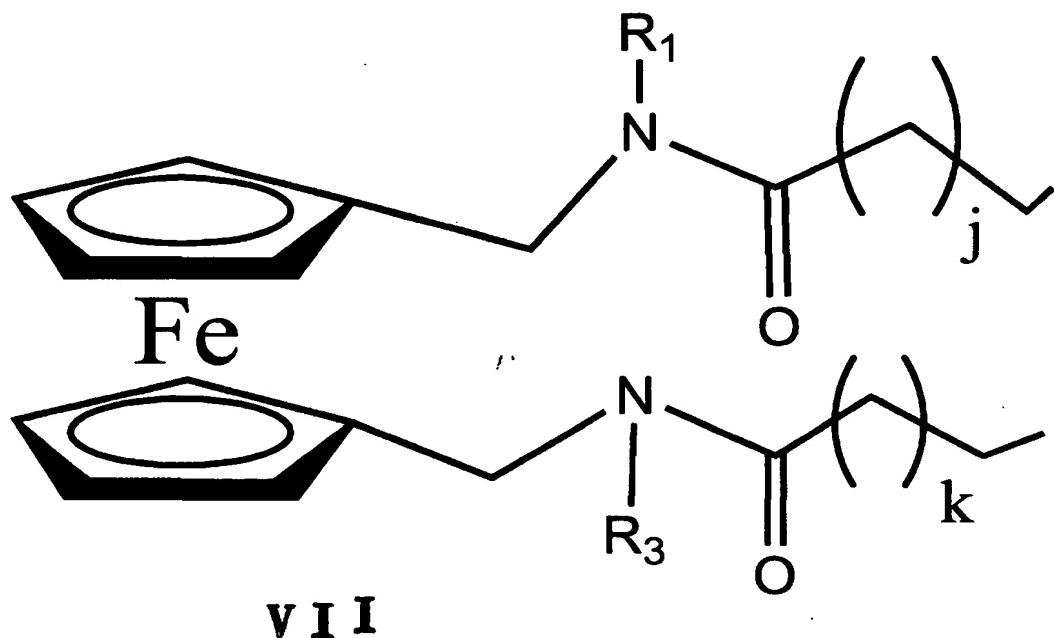
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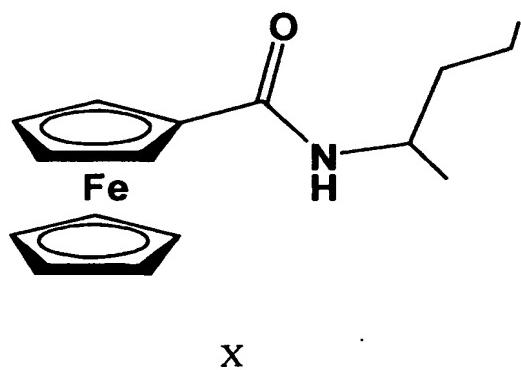
[Chemical Formula 7]



[Chemical Formula 8]



[Chemical Formula 9]



5

wherein R<sub>1</sub> and R<sub>3</sub> represent a hydrogen atom or alkyl; "j" and "k" represent the same or different integer of from

0 to 5. R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are preferably alkyl having one or several carbon atoms, such as a methyl group.

Specific examples of the above compounds include those

5 represented by the formulae (VIII or 1a), (IX), (1b), (1c), (2) and (3):

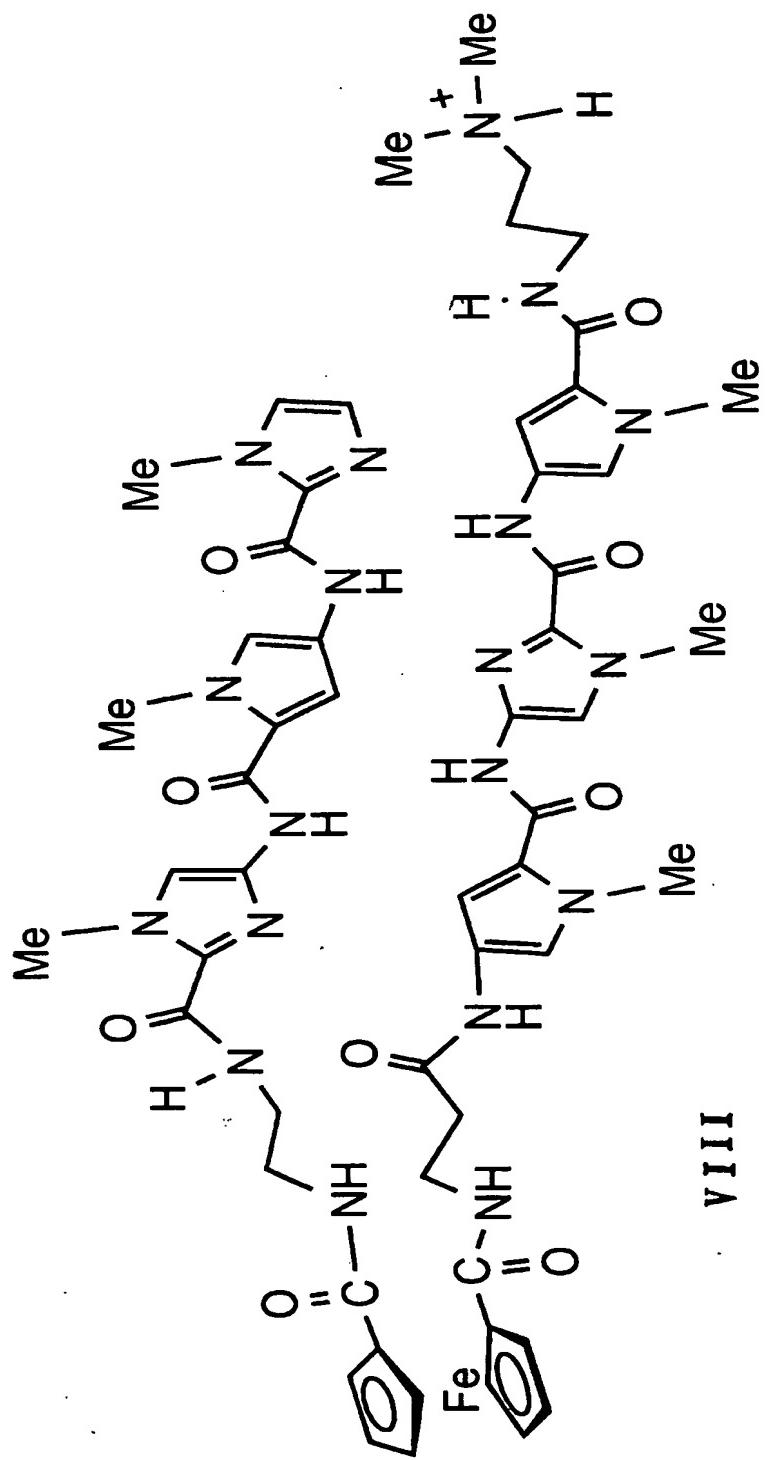
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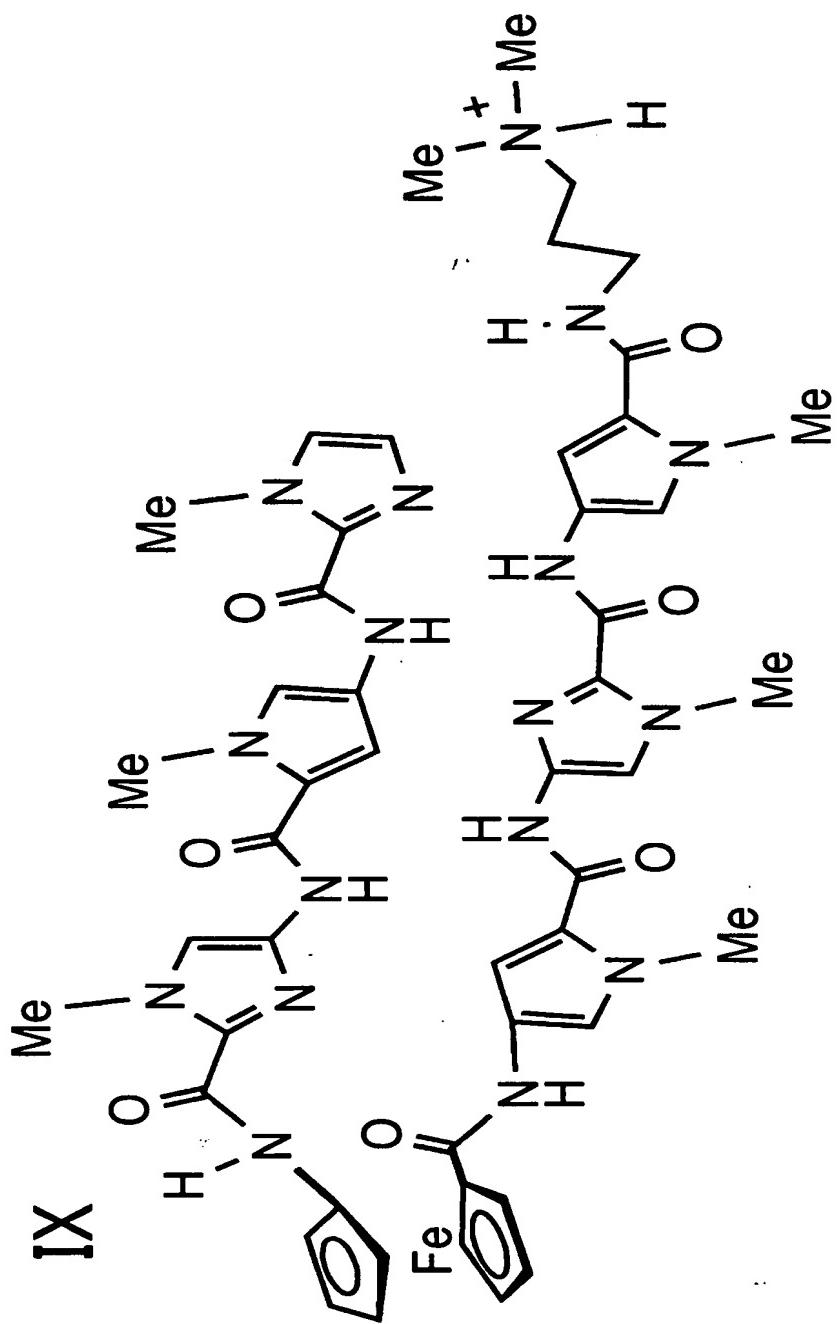
25

[Chemical Formula 10]

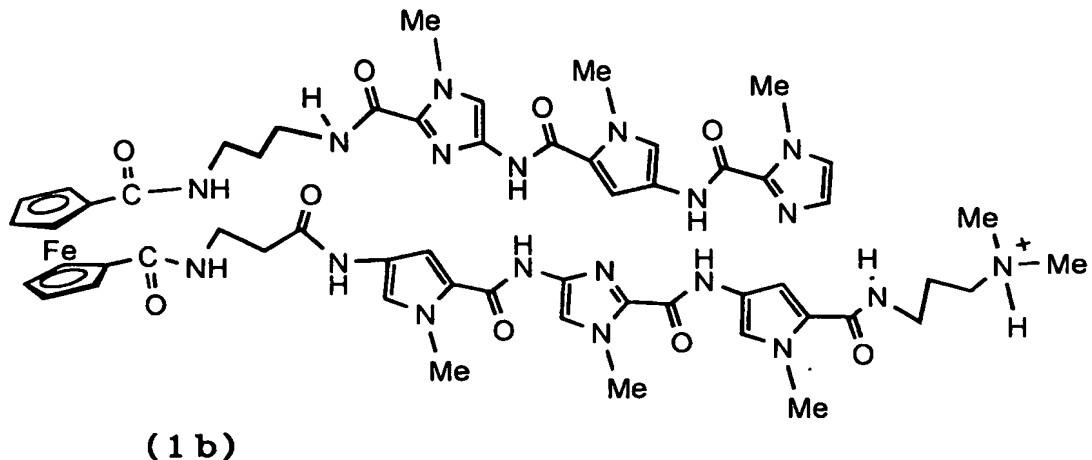


VIII

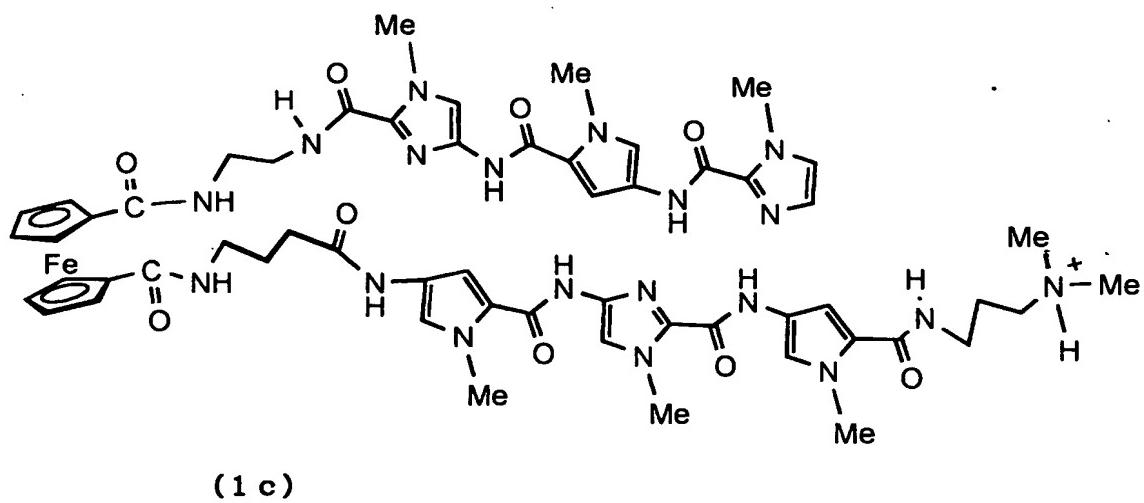
[Chemical Formula 11]



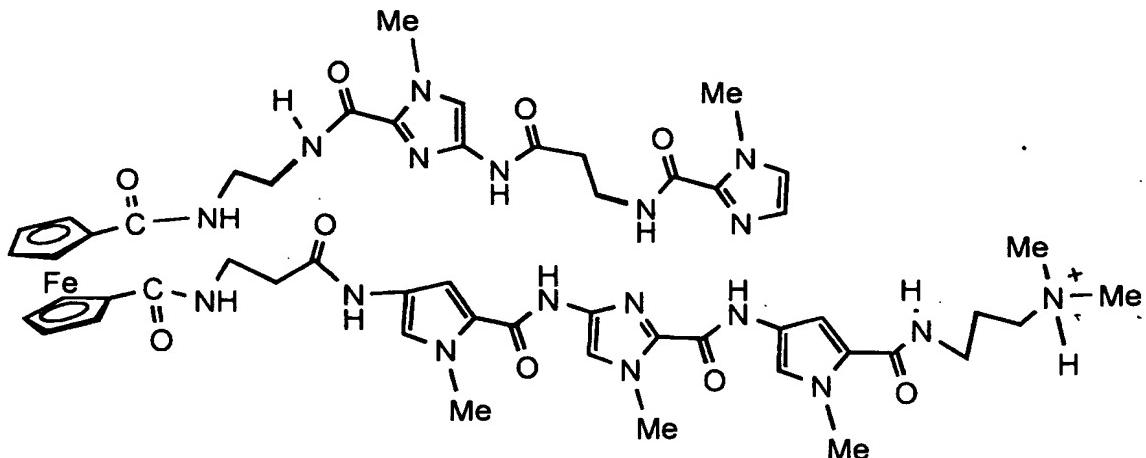
[Chemical Formula 12]



[Chemical Formula 13]

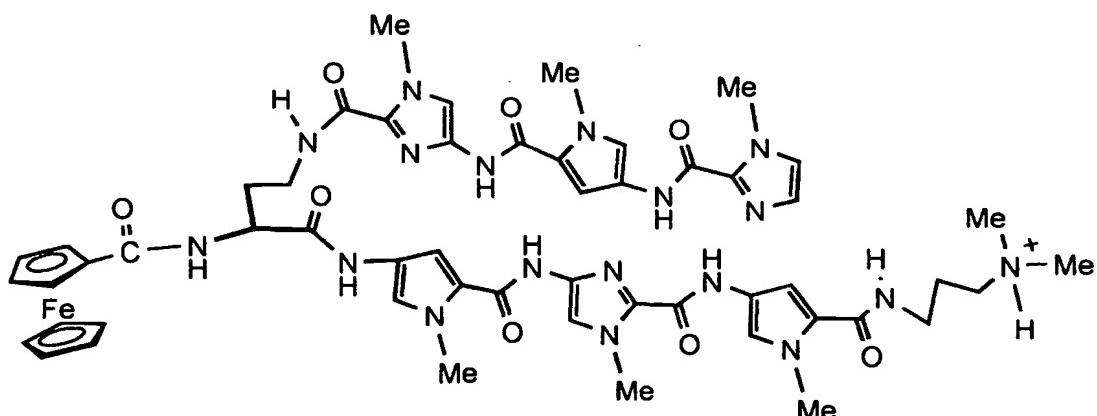


[Chemical Formula 14]



(2)

[Chemical Formula 15]



(3)

The compounds according to the present invention may be synthesized by the processes described in the following examples. Reaction conditions that are not specifically described in the present specification may be optionally 5 and easily adopted by those skilled in the art.

Accordingly, the present invention relates as a second aspect to a method for the production of the ferrocene compound according to the present invention, comprising 10 a condensation step with the use of ferrocene methyl dicarboxylate, aminoferrocene methyl carboxylate or ferrocene carboxylic acid as a starting material. The present method are exemplified in reaction schemes represented by Chemical Formulae 16 to 21, and include the 15 methods comprising the steps described in the examples of the present specification.

The present invention relates as a third aspect to a ligand consisting of the ferrocene compound according to the 20 present invention for sequence-specific detection of double-stranded nucleic acid molecules.

The present invention relates to as a forth aspect to a method for the electrochemical detection of 25 double-stranded nucleic acid molecules and an apparatus or device for the electrochemical detection with the use of a compound that can sequence-specifically bind to the double-stranded nucleic acid molecules, such as the above

ligand.

#### Advantages of the invention

- 5 By using the compound that can sequence-specifically bind to the double-stranded nucleic acid molecules, such as the ferrocene compound represented by the general formula (I), it is possible to lower the electrochemical signal/noise ratio (S/N) in the electrochemical detection. As a result,
- 10 Significant improvement of the detection sensitivity (precision) is attained, enabling a quantitative determination of an ultratrace amount of the double-stranded nucleic acid molecules.

#### 15 Brief description of drawings

Fig. 1 shows CD profile in the titration of the 15-mer DNA prepared in Example 2. The reaction was carried out in 10 mM sodium cacodylate (pH 6.9) containing 10mM KCl, 10  
20 mM MgCl<sub>2</sub>, 5 mM CaCl<sub>2</sub>.

Fig. 2 shows CD profile in the titration of the 11-mer DNA prepared in Example 5. The titration of the double-stranded DNA (5μM) with the compound (1a) (0-1.8  
25 equivalent) (a), with the compound (3) (0-1.6 equivalent) (b), and with the compound (2) (0-2.0 equivalent) (c), respectively.

Fig.3 shows the electrochemical properties of the present compounds, which were obtained in an experiment with a cyclic voltammetry (CV) in Example 6. The experiment was carried out by using the compound of the present invention 5 (5 mM) in N,N-dimethylformamide containing 0.2 M NaClO<sub>4</sub>. Reference electrode:AgCl<sub>2</sub>; Counter electrode:Pt; operating Electrode:Pt.

#### BEST MODE FOR CARRYING OUT THE INVENTION

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The term "nucleic acid" in the present specification means both DNA and RNA, and the double-stranded nucleic acid molecules include DNA-DNA and DNA-RNA and the like. For example, mRNA, cDNA that has been prepared by reverse 15 transcription, and their fragment may be used as a sample (target) nucleic acid molecule. On the other hand, cDNA or its fragment, synthesized DNA and the like may be used as a probe nucleic acid molecule. They are well known for those skilled in the art, and may be selected depending 20 a kind of the method or apparatus of the electrochemical detection and the like.

High sequence-specificity may be attained in the electrochemical detection method according to the present 25 invention by designing the present compound so that each pair of "V" and "X" located in the general formula (I) at a position corresponding to G/C and A/T (U) base pairs in subject double-stranded nucleic acid molecules shall be

composed of imidazole derivative/pyrrole derivative and pyrrole derivative/pyrrole derivative, respectively.

Measurement principle in the electrochemical detection  
5 method according to the present invention is hybridization  
between the nucleic acid molecules, which may be carried  
out any method or operation known for those skilled in the  
art. For example, a method using DNA microarray (DNA  
chip) )may be listed as that with the use of hybridization  
10 on solid phase. The apparatus or device for the  
electrochemical detection per se such as an electrochemical  
chip or ECA chip is well known for those skilled in the  
art, which may be used in the method of the present invention.  
Said apparatus or device may be alternatively manufactured  
15 in accordance with a method well known for those skilled  
in the art.

The electric signal to be detected includes currency,  
electroconductivity, electric potential, electric  
20 capacity, inductance, and impedance, which may be detected  
with the apparatuses such as a cyclic voltammetry (CV),  
differential pulse voltammetry (DPV), linear sweep  
voltammography and potentiostat.

25 The method for electrochemical detection of  
double-stranded nucleic acid molecules according to the  
present invention with the use of the ligand of the present  
invention for the sequence-specific detection of

double-stranded nucleic acid molecules may be utilized in various methods for detection based on the hybridization of the nucleic acids, including polymorphism analysis such as SNP, determination of base sequences, analysis of gene mutation, monitoring of gene expression and the like.

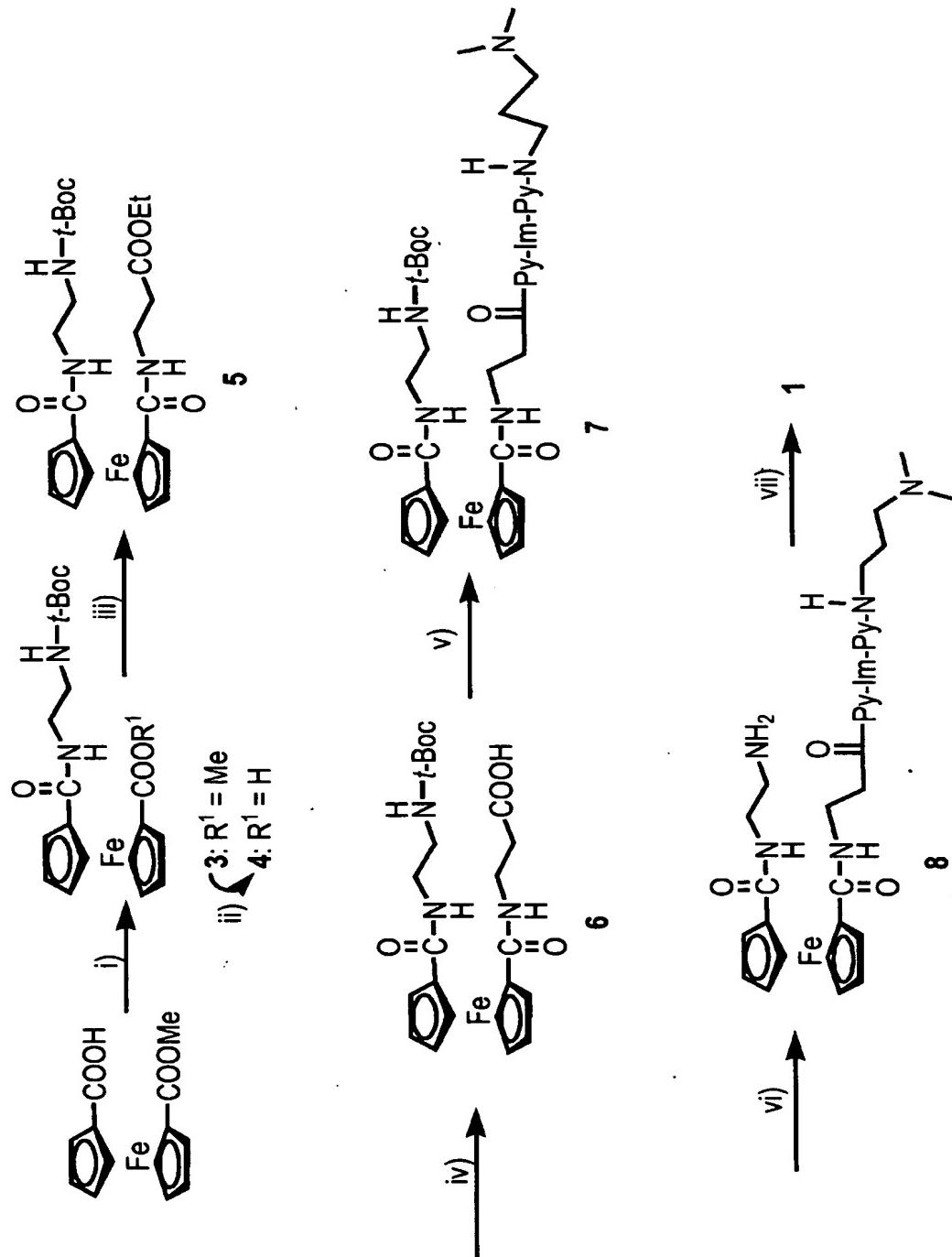
EXAMPLE

The present invention will be explained more in detail in line with the examples. Those skilled in the art may easily conceive other various examples having the features of the present invention based on the technical common knowledge in the art and on the description of the present specification, which shall therefore fall within the scope of the present invention as well.

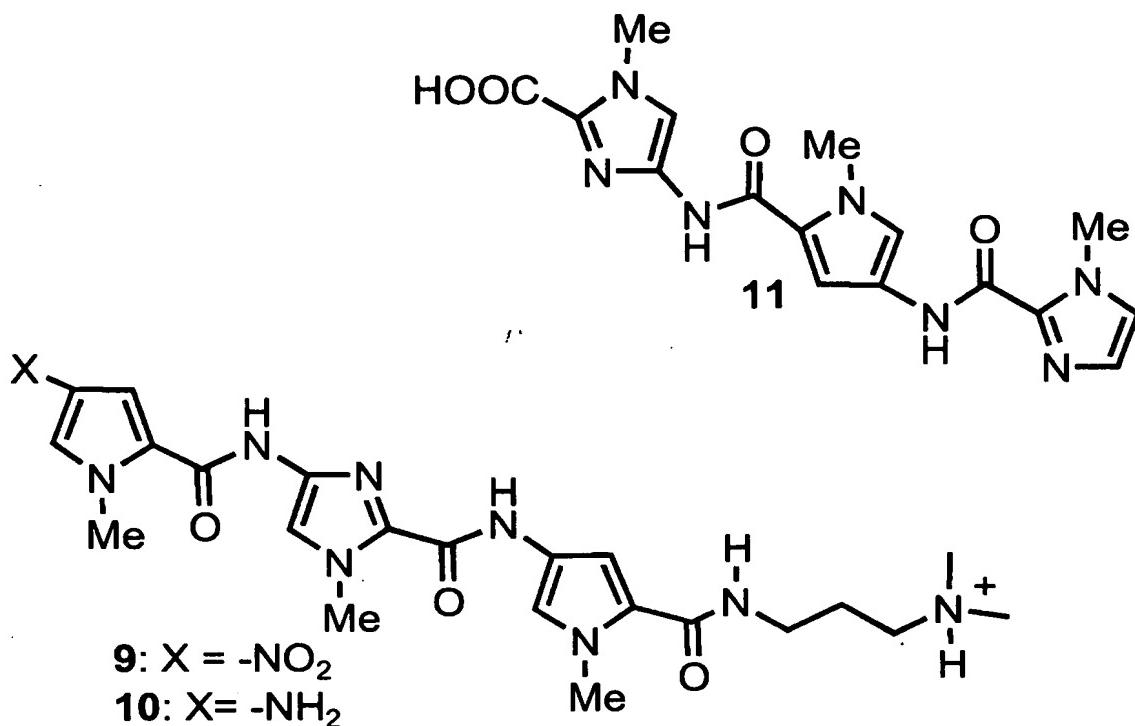
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Example 1: Synthesis of the compound of the formula (VIII)  
 [Chemical Formula 16]



[Chemical Formula 17]



The structure of the above compounds was selected in view  
of availability of starting materials and chemical  
stability of the final and intermediate products. The  
sequence of the PIPA region was designed so as to bind to  
a target single base mutation in the human genome, which  
may be a factor determining interferon-resistance in a  
patient, i.e., CGC/GCG sequence.

10

Ferrocene methyl dicarboxylate (2) was condensed with  
N-t-butoxycarbonyl-1,3-propanediamine by means of

2-chloro-1-methyl pyridinium iodide (CMPI) (Bald, E.; Saigo, K.; Mukaiyama, T. Chem. Lett. 1975, 1163-1166) with a yield of 87%. The resulting compound (3) was converted into its carboxylic acid (4) by the treatment with an  
5 alkaline aqueous solution, which was then condensed with ethyl 3-aminopropionate to give a ferrocene-binding unit (5) with a yield of 62%.

Ethyl ester of the above compound (5) was removed by the  
10 treatment with an alkaline aqueous solution for the introduction of pyrrole-imidazole moiety (yield: 93%). A free carboxyl group of the compound (6) was bound to 3-aminopyrrole derivative (10) that had been prepared in situ by hydrogenation of 3-nitropyrrole derivative (9) with  
15 CMPI to give a compound (7) (yield: 62%). The resulting compound (7) was further hydrolyzed by the treatment with 10% TFA in CH<sub>2</sub>Cl<sub>2</sub> to give its amine (8) (yield: 71%).

The compound (8) (187.6 mg, 0.4777 mmol) was dissolved in  
20 methylene chloride (4.77 ml), to which were added diisopropylethylamine (0.162 ml, 0.947 mmol) and O-(benzotriazole-yl)-N,N,N',N'-tetramethyl uronium hexafluorophosphate (361.8 mg, 0.954 mmol) as a condensing agent (Dourtoglou, V.; Ziegler, J.-C.; Gross, B.  
25 Tetrahedron Letters 1978, 15, 1269-1272, et al.), and stirred for 30 min at a room temperature. Methylene chloride (4.8 ml) dissolving a carboxylic acid (11) (400 mg, 0.477 mmol) prepared separately (Baird, E. E., Dervam,

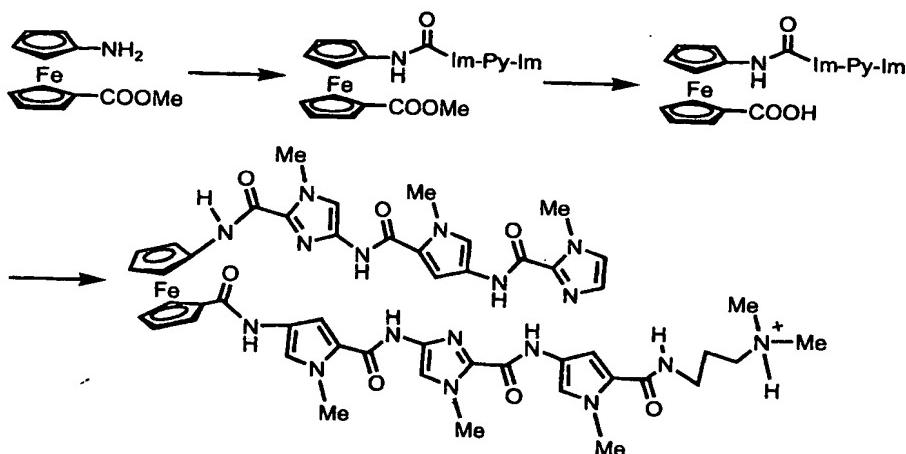
P.B. J. Am. Chem. Soc., 1996, 118, 6414-6416) was added to the above mixture and stirred for one hour. After the resulting mixture was washed with water, an organic layer was collected and dried over anhydrous sodium sulfate, so  
5 that the solvent was distilled out under a reduced pressure. The residue was purified with silica gel chromatography to give a final product (349 mg, 61%). The structure of the product was confirmed by <sup>1</sup>H-NMR and <sup>13</sup>C-NMR.

10     <sup>1</sup>H-NMR(DMSO-d6) δ 1.59-1.65( 2H, m ), 2.15( 6H, s ),  
      2.26-2.28( 2H, t, J =7.1 Hz), 2.56-2.59( 2H, t, J = 7.0  
      Hz ), 3.18-3.22( 2H, m,), 3.37-3.50( 6H,m), 3.81( 3H, s  
      ), 3.83( 3H, s ), 3.85( 3H, s ),3.94( 3H, s ), 3.97( 3H,s  
      ), 3.99( 3H, s ), 4.27-4.30( 4H, m), 4.73( 4H, s ), 6.88(1H,  
15     d, J = 1.7Hz ), 6.94(1H, d, J = 1.5 Hz), 7.02( 1H, s ), 7.16(1H,  
      d, J = 1.7 Hz), 7.19(1H, d, J = 1.5 Hz), 7.26(1H, d, J =  
      1.7 Hz), 7.35-7.36( 2H, m), 7.46(1H, s ), 7.49( 1H, s ),  
      7.86-7.87( 1H, t, J = 5.6 Hz), 7.91-7.93( 1H, t, J =5.5  
      Hz ), 7.98-7.80( 1H, t, J = 5.5 Hz), 8.05-8.08( 1H, t, J  
20     = 5.7 Hz ),9.77( 1H, s ), 9.88( 1H, s ), 10.03( 1H, s ),  
      10.06( 1H, s ), 10.19( 1H, s )

13C-NMR(DMSO-d6) δ 27.2, 35.1, 35.1, 35.3, 36.0, 36.1, 36.2,  
      36.4, 36.5,37.4, 38.8, 39.0, 45.3, 57.3, 69.8, 71.7, 71.8,  
25     77.9, 78.0, 79.3, 104.1,104.8, 106.1, 114.6, 114.9, 118.0,  
      119.4, 119.6, 121.4, 1221.7, 122.2,122.3, 122.4, 123.5,  
      126.6, 127.2, 134.1, 134.4, 136.2, 138.9, 155.9,  
      156.3,158.9, 158.9, 159.2, 161.2, 168.2, 168.6, 169.0MS

m/z calcd for C<sub>55</sub>H<sub>66</sub>FeN<sub>19</sub>O<sub>9</sub><sup>+</sup> : 1192.46403 , found  
1192.37089.

Similarly, the compound of the formula (IX) was synthesized  
5 in accordance with the following reaction scheme. The  
structure of the product was confirmed by <sup>1</sup>H-NMR and <sup>13</sup>C-NMR.  
[Chemical Formula 18]



10

<sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) δ 1.57-1.63 (2H, m), 2.13 (6H, s),  
2.22-2.25 (2H, t, J = 6.9 Hz), 3.16-3.20 (2H, dd, J = 6.9 Hz),  
3.76 (3H, s), 3.79 (3H, s), 3.82 (3H, s), 3.85 (3H, s), 3.96 (3H,  
s), 3.99 (3H, s) 4.04 (3H, t, J = 1.8 Hz), 4.35 (3H, t, J  
15 = 1.8 Hz), 4.85 (3H, t, J = 2.0 Hz), 4.95 (3H, t, J = 1.8  
Hz), 6.88 (1H, d, J = 1.7 Hz), 6.97 (1H, d, J = 1.7 Hz), 7.03  
(1H, d, J = 1.0 Hz), 7.07 (1H, d, J = 1.7 Hz), 7.19-7.20  
(2H, m), 7.37 (1H, s), 7.38 (1H, s), 7.49 (1H,  
s), 7.52 (1H, s), 8.07-8.10 (1H, t, J = 5.6 Hz), 9.13 (1H  
20 , s), 9.51 (1H, s), 9.90 (1H, s), 10.16 (1H, s), 10.25

(1H, s ), 10.35 (1H, s )

<sup>13</sup>C-NMR (DMSO-d6) δ 27.3, 33.6, 35.1, 35.3, 36.2, 36.4, 36.5,  
37.4, 45.4, 57.4, 61.9, 65.7, 69.3, 71.1, 78.3, 79.4, 96.1,  
5 104.1, 105.4, 106.1, 114.9, 115.0, 118.0, 119.7, 119.8,  
121.3, 121.7, 121.8, 122.3, 122.4, 123.5, 126.6, 127.2,  
133.9, 134.3, 136.2, 136.3, 138.9, 155.9, 156.3, 156.9,  
158.9, 158.9, 161.2, 165.7

MS m/z calcd for C<sub>49</sub>H<sub>56</sub>FeN<sub>17</sub>O<sub>7</sub><sup>+</sup> : 1050.38980 , found  
10 1050.40297.

Example 2: Properties of the compound of the formula (VIII)  
as a ligand

15 The binding of the above compound to a target DNA  
(TTTCTGCGGCCGGAG/CTCCGGCCGCAGAAA : Tm = 67.1°C) was  
detected with CD spectrum in order to confirm its utility  
as a ligand for the double-stranded helical DNA. The  
results in Fig.1 show a CD profile in the titration of the  
20 above 15-mer DNA with 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and  
1.4 equivalents of the present compound. It is well known  
that elliptical polarization at 300 - 360 nm was induced  
by the interaction with a minor groove (Pilch, D.S.; Poklar,  
N.; Baird, E.E.; Dervan, P.B.; Breslauer, K.J. Biochemistry,  
25 2002, 38, 2143-2151). As a result, the addition of the  
solution of the target DNA (5.0 μM) caused  
concentration-dependent increase of a positive cotton  
effect with an isosbestic point at 292 nm.

Next, the present compounds were further synthesized in accordance with the following reaction schemes:

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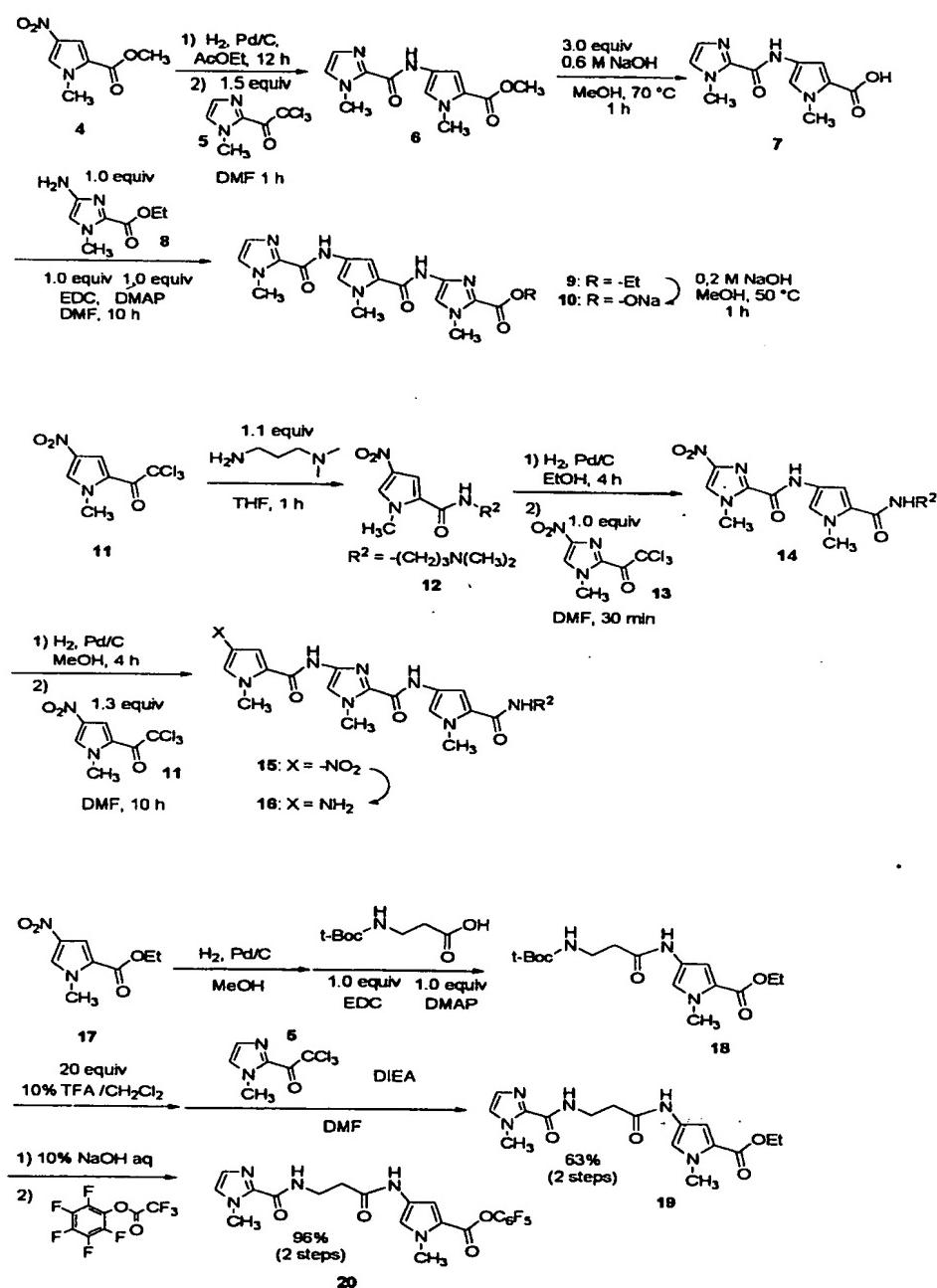
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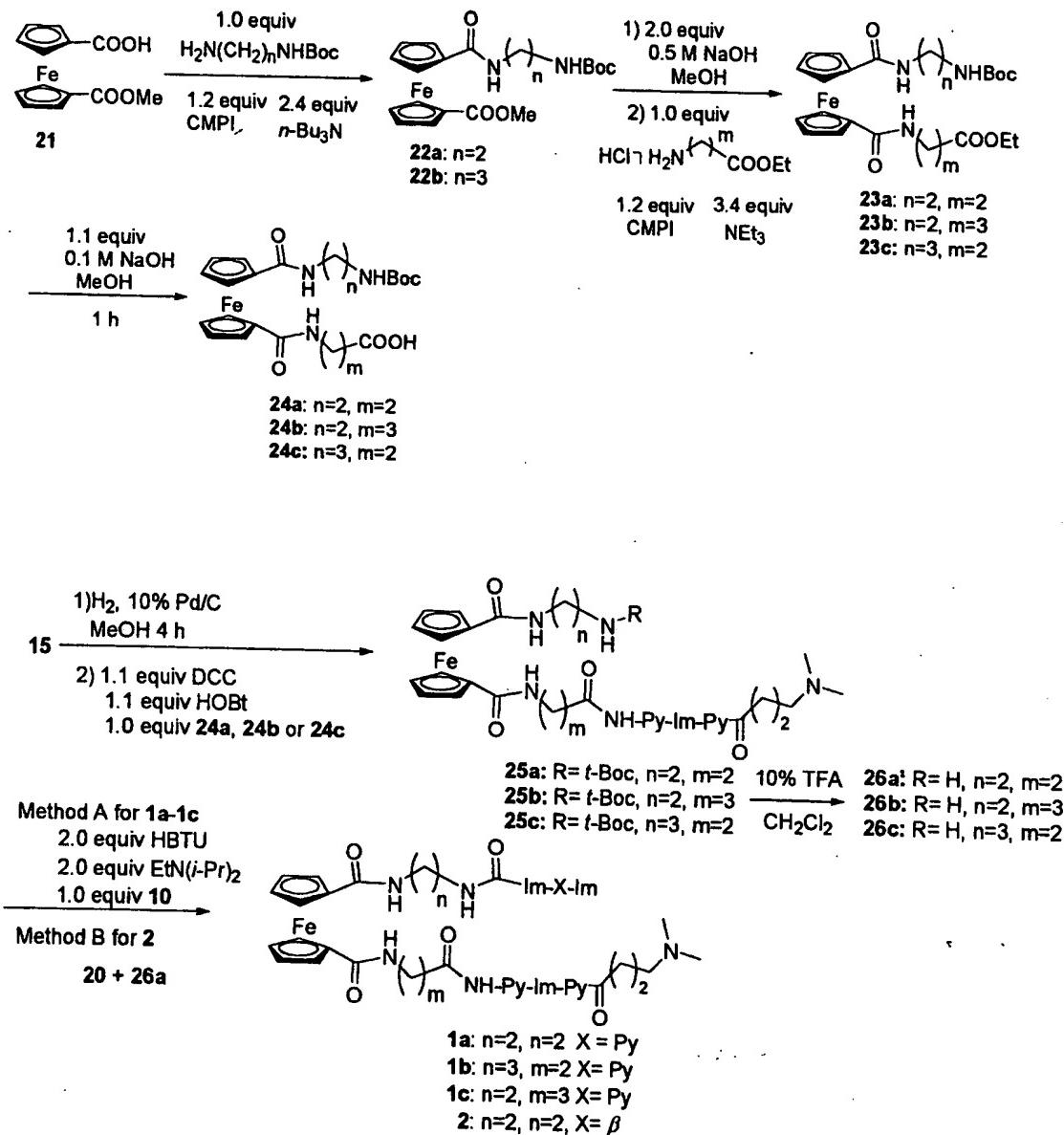
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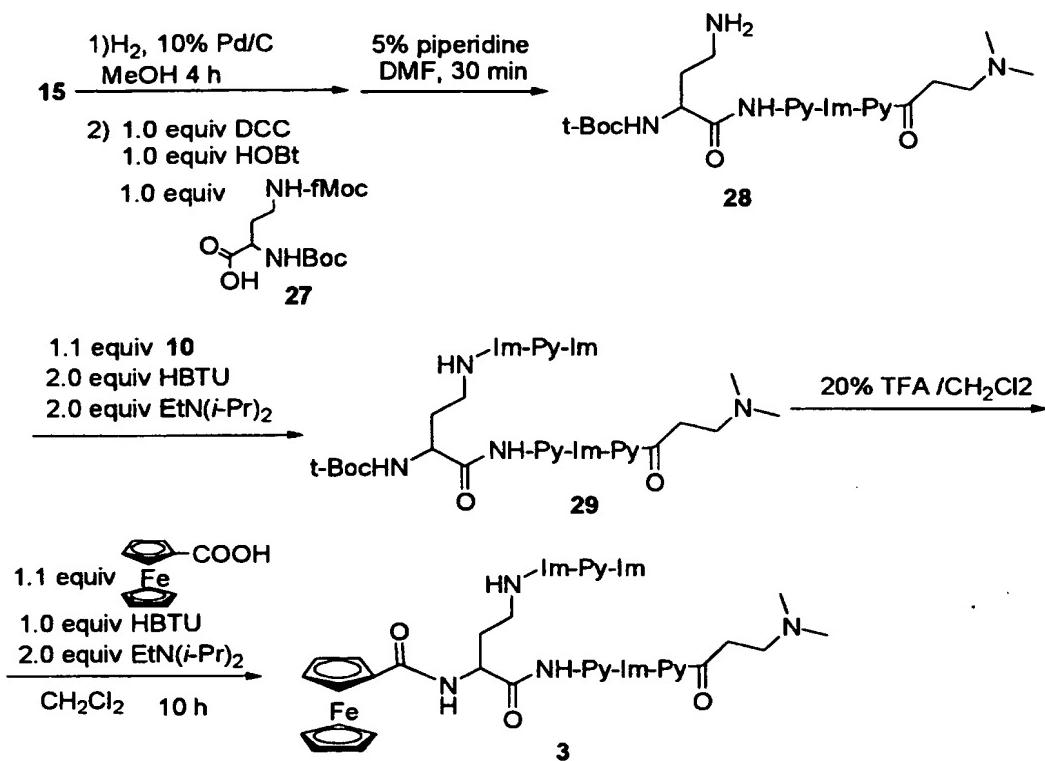
## [Chemical Formula 19]



## [Chemical Formula 20]



[Chemical Formula 21]

5 Synthesis Example 1 of the material compound:1-Methyl-4-(1-methylimidazole-2-carboxamido)pyrrole-2-carboxylic acid (7)

The compound 6 (8.8g, 33.6 mmol) prepared from the compound 10 4 (Baird, E. E.; Dervan, P. B. *J. Org. Chem.* 1996, 118, 6141-6146) and the compound 5 (Nishiwaki, E.; Tanaka, S.; Lee, H.; Shibuya, M. *Heterocycles* 1988, 8, 1945-1952) by the methods described in the articles was dissolved in ethanol (234mL), mixed with 0.6 M aqueous solution of sodium

hydroxide (168 ml, 110.7 mmol) and stirred for one hour at 70°C. After being cooled to a room temperature, the resulting solution was mixed with hydrochloric acid (3N), so that white powder was generated at pH 3.0, which was 5 then filtered to give the compound 9 (8.1 g, 97%).

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 3.82 (3H, s), 3.97 (3H, s), 6.97 (1H, d, J = 2.0 Hz), 7.03 (1H, s), 7.38 (1H, s), 7.47 (1H, d, J = 2.0 Hz), 10.48 (1H, s), 12.19 (1H, s); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ 35.3, 10 36.4, 109.1, 119.9, 120.7, 122.1, 126.6, 127.2, 138.8, 156.3, 162.1. Anal. Calcd. for C<sub>11</sub>H<sub>12</sub>N<sub>4</sub>O<sub>3</sub>: C, 53.22; H, 4.89; N, 22.57. Found: C, 52.45; H, 4.72; N, 22.34.

Synthesis Example 2 of the material compound:

15 2-Ethoxycarbonyl-1-methyl-4-{4-(1-methylimidazole-2-carboxamido)-1-methypyrrole-2-carboxamido}imidazole (9)

The compound 7 was dissolved in methanol (94.4 ml), mixed with 10 % Pd/C (1.18 g) and stirred for four hours under 20 hydrogen atmosphere. 10% Pd/C was removed from the reaction system with cerite filtration, so that the solvent was distilled out under a reduced pressure.

Dimethylformamide (10 ml) was added to the residue and distilled out again under a reduced pressure. The residue 25 was dissolved in dimethylformamide (10 mL) again, and mixed with the compound 8 (5.9 g, 23.6 mmol) (Baird, E. E.; Dervan, P. B. *J. Org. Chem.* **1996**, *118*, 6141-6146), EDC (9.05 g, 47.2 mmol) and dimethylaminopyridine (288 mg, 2.36 mmol),

and stirred for 12 hours at a room temperature.  
Re-precipitation treatment with chloroform and ethyl acetate gave the compound 9 as white powder (5.9 g, 63%).

5       $^1\text{H}$ NMR (DMSO-d<sub>6</sub>)  $\delta$  1.28-1.30 (3H, t, J = 7.1 Hz), 3.85 (3H, s), 3.93 (3H, s), 3.98 (3H, s), 4.25-4.29 (2H, dd, J = 7.1 Hz), 7.03 (1H, d, J = 0.7 Hz), 7.22 (1H, d, J = 2.0 Hz), 7.38 (1H, s), 7.38 (1H, s), 7.66 (1H, s), 10.35 (1H, s), 10.74 (1H, s);  $^{13}\text{C}$ NMR (DMSO-d<sub>6</sub>)  $\delta$  14.3, 35.3, 35.7, 36.5, 60.8, 10 106.4, 115.6, 119.8, 121.6, 122.2, 126.5, 127.2, 131.0, 138.0, 139.0, 156.3, 158.7, 158.9: Anal. Calcd. for C<sub>18</sub>H<sub>12</sub>N<sub>4</sub>O<sub>3</sub>·H<sub>2</sub>O: C, 53.22; H, 4.89; N, 22.57. Found: C, 50.88; H, 4.88; N, 23.13.

15      Synthesis Example 3 of the material compound:  
Sodium 1-methyl-4-{4-(1-methylimidazole-2-carboxamido)-1-methypyrrole-2-carboxamido}imidazole-2-carboxylate  
(10)

20      The compound 9 (3.0 g, 7.5 mmol) was dissolved in methanol (37.6 ml), mixed with aqueous solution of sodium hydroxide (37.6 ml, 7.89 mmol) and stirred for one hour at 50°C. After being cooled to a room temperature, the resulting solution was mixed with hydrochloric acid (1N) to reach pH 2.0, and 25 then mixed with isopropylalcohol to give the white powder, which was then filtered to give the compound 10 (2.85 g, 94%).

<sup>1</sup>HNMR (DMSO-d<sub>6</sub>) δ 3.86(3H, s), 3.92(3H, s), 4.01(3H, s), 7.23(1H, s), 7.25(1H, s), 7.40(1H, s), 7.52(1H, s), 7.61(1H, s), 10.70(1H, s), 10.71(1H, s); <sup>13</sup>CNMR (DMSO-d<sub>6</sub>) δ 25.6, 35.7, 36.5, 106.2, 115.3, 119.9, 121.3, 122.4, 125.1, 126.6, 131.9, 137.3, 138.1, 154.7, 158.8, 160.1. MS m/z calcd for C<sub>16</sub>H<sub>18</sub>N<sub>7</sub>O<sub>4</sub><sup>+</sup>: 372.14203, found 394.11781[M+Na]<sup>+</sup>.

Synthesis Example 4 of the material compound:

2-(3-Dimethylaminopropyl)aminocarbonyl-1-methyl-4-nitr  
10 opyrrole (12)

The compound 11 (25 g, 92 mmol) disclosed in the article (Nishiwaki, E.; Tanaka, S.; Lee, H.; Shibuya, M. *Heterocycles* **1988**, *8*, 1945-1952) was added dropwise to THF (10 ml) dissolving 3-dimethylaminopropylamine (12.6 g, 101 mmol) at 0°C. After being heated to a room temperature, the resulting solution was stirred for one hour, and the solvent was distilled out. Re-crystallization of the residue with methanol gave the compound 12 (19.6 g, 84%).

20

<sup>1</sup>HNMR (DMSO-d<sub>6</sub>) δ 1.6(t, J=7.1Hz), 2.1(6H, s), 2.2(2H, t; J=7.1Hz), 3.2(2H, q, J=7.1Hz) 3.9(3H, s), 7.9(1H, s), 8.1(1H, s), 8.4(1H, t, J=5.5Hz).

25 Synthesis Example 5 of the material compound:

1-Methyl-4-(1-methyl-4-nitroimidazole-2-carboxamido)-2-(3-dimethylaminopropylaminocarbonyl)pyrrole (14)

The compound 12 (2.8 g, 10.9 mmol) was dissolved in methanol (44 mL), mixed with 10 % Pd/C (545 mg) and stirred under hydrogen atmosphere for four hours at a room temperature. 10% Pd/C was filtered out, and the solvent was distilled out. The residue was dissolved in dimethylformamide (10 mL), followed by azeotropy to reduce its volume by half so as to remove methanol. The known compound 13 (3.0 g, 10.9 mmol) dissolved in DMF (10 ml) was added to the resulting solution at 0°C. After being heated to a room temperature, the solvent was distilled out and the residue was washed with 2-propanol to give the compound 14 (3.68 g, 90 %).

<sup>1</sup>HNMR (DMSO-d<sub>6</sub>) δ 1.6 (2H, t, J=7.1Hz),  
2.1(6H,s), 2.2(2H,t;J=7.1Hz), 3.2(2H,m), 3.8(3H,s),  
4.0(3H,s), 7.0(1H,d,J=1.7Hz), 7.3(1H,d,J=1.7Hz),  
8.1(1H,t,J=5.5Hz), 8.6(1H,s), 10.8(1H,s).

Synthesis Example 6 of the material compound:  
20 1-Methyl-4-{1-methyl-4-(1-methyl-4-nitropyrole-2-carbo-  
xamido)imidazole-2-carboxamido}-2-(3-dimethylaminoprop-  
ylaminocarbonyl) pyrrole (15)

The compound 14 (3.6 g, 9.6 mmol) was dissolved in methanol (38 mL), mixed with 10 % Pd/C (481 mg) and stirred under hydrogen atmosphere for four hours at a room temperature. 10% Pd/C was filtered out, and the solvent was distilled out. The residue was dissolved in DMF (10 mL), followed

by azeotropy to reduce its volume by half so as to remove methanol. The compound 11 (2.6 g, 9.6 mmol) dissolved in DMF (10 ml) was added to the resulting solution at 0 °C. After being heated to a room temperature, the solvent 5 was distilled out and the residue was washed with 2-propanol to give the compound 15 (3.5 g, 74 %).

<sup>1</sup>HNMR (DMSO-d<sub>6</sub>) δ 1.74-1.77(2H, t, J=6.1Hz), 2.27(6H, s), 2.43-2.46(2H, t, J=6.1Hz), 3.49-3.53(2H, m), 3.86(3H, s), 10 4.05(6H, s), 6.57(1H, s), 7.16(1H, s), 7.27(1H, s), 7.44(1H, s), 7.61(1H, s), 7.69(1H, s), 7.90(1H, m), 8.58(1H, s), 9.25(1H, s).

Synthesis Example 7 of the material compound:

15 2-Ethoxycarbonyl-1-methyl-4-{3-(tert-butoxycarbonylamino)propanamido}pyrrole (18)

The compound 17 (546 mg, 2.7 mmol) disclosed in Baird, E. E.; Dervan, P. B. *J. Org. Chem.* **1996**, *118*, 6141-6146 was dissolved in methanol (11 ml), mixed with 10 % Pd/C (137 mg) and stirred under hydrogen atmosphere for one hour and half at a room temperature. 10% Pd/C was filtered out, and the solvent was distilled out. The residue was dissolved in methylene chloride, mixed with 25 N-(tert-butoxycarbonyl)-β-alanine (518 mg, 2.7 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarboxydiimide hydrochloride (578 mg, 3.0 mmol), and 1-hydroxybenzotriazole (407 mg, 3.0 mmol) and stirred for

two hours at a room temperature. The resulting solution was extracted with ethyl acetate (50 ml) and water (50 mL), dehydrated over sodium sulfate and concentrated under a reduce pressure. The resulting residue was purified with 5 silica gel column (11 g) to give the compound 18 (510 mg, 56%).

<sup>1</sup>HNMR (DMSO) δ 1.27-1.30 (2H, t, J=7.2Hz), 1.36(9H, s),  
2.40-2.43(2H, t, J=7.2z), .3.16-3.20(2H, m), 3.90(3H, s),  
10 4.23-4.28(2H, q, J=7.1Hz), 6.78(1H, m), 7.51(1H, s),  
10.6(1H, s).

Synthesis Example 8 of the material compound:

2-Ethoxycarbonyl-1-methyl-4-{3-(1-methyimidazole-2-  
15 carboxamido) propanamido}pyrrole (19)

The compound 18 (510 mg, 1.5 mmol) was mixed with 10% trifluoroacetic acid/methylene chloride (24 mL, 30 mmol) and stirred for one hour at a room temperature. The 20 resulting solution was washed with water (15 mL), dehydrated over sodium sulfate, filtered and concentrated. The resulting residue was dissolved in DMF, mixed with N,N'-diisopropylamine (128 µl, 0.75 mmol) and the compound 5 (490 mg, 1.8 mmol) and stirred for two hours at a room 25 temperature. The resulting solution was extracted with chloroform (30 ml) and water (20 mL), dehydrated over sodium sulfate and concentrated. Re-precipitation of the resulting residue from chloroform and diisopropylether

gave the compound 19 (475 mg, 91 %).

<sup>1</sup>HNMR(DMSO-d<sub>6</sub>) δ 1.26-1.28 (3H, t, J=7.1Hz),  
2.53-2.56(2H, t, J=6.8Hz), 3.45-3.49(2H, m), 3.89(3H, s),  
5 3.92(3H, s), 4.22-4.26(2H, m), 6.94(1H, s), 7.31(1H, s),  
7.53(1H, s), 8.34(1H, m), 10.7(1H, s).

Synthesis Example 9 of the material compound:

2-pentafluorophenoxy carbonyl-1-methyl-4-{3-(1-methyimi  
10 dazole-2-carboxamido)propanamido}pyrrole (20)

The compound 19 (65 mg, 0.19 mmol) was dissolved in methanol (19 mL), mixed with aqueous solution of sodium hydroxide (0.4 M) (1.4 mL, 0.56 mmol) and stirred for one hour at 15 50°C. After being cooled to a room temperature, the solution was adjusted to pH 2 with 1 M hydrochloric acid. The precipitated powder was washed with 2-propanol, and the solvent was distilled out. The residue was dissolved in DMF (1.3 mL), mixed with 20 pentafluorophenyltrifluoromethyl acetate (68 μL, 0.39 mmol) and pyridine (32 μL, 0.39 mmol), and stirred under argon atmosphere for one hour at a room temperature. The solution was washed with 0.1 M hydrochloric acid (10 mL x 3), 5% aqueous solution of sodium hydrogen carbonate (10 mL) and saturated saline (10 mL), and dehydrated over sodium sulfate. The residue was concentrated under a reduced pressure to give the compound 20 (88 mg, 96 %).

<sup>1</sup>HNMR (DMSO-d<sub>6</sub>) δ 2.58-2.61(2H, t, J=7.0Hz),  
3.49-3.53(2H, m), 3.94(3H, s), 3.98(3H, s),  
6.96(1H, d, J=1.0Hz), 7.37(1H, d, J=0.6Hz),  
8.37-8.39(1H, t, J=6.0Hz), 10.9(1H, s).

5

Synthesis Example 10 of the material compounds: a general synthesis method of ferrocene derivatives (22a) and (22b)

The compound 21 (1.0 g, 3.47 mmol) was dissolved in methylene  
10 chloride (35 ml), and mixed with  
N-tert-butoxycarbonylethylenediamine (0.56 g, 3.47 mmol),  
n-tributylamine (2.0 mL, 8.3 mmol) and 2-chloro-1-methyl  
pyridinium iodide (1.1 g, 4.16 mmol). The mixture was  
washed with water (20 ml) and saturated saline, dehydrated  
15 over sodium sulfate and concentrated under a reduced  
pressure. The residue was purified with column  
chromatography to give the compound (22a) (1.3 g, 87 %).

22a:

20 <sup>1</sup>HNMR (DMSO-d<sub>6</sub>) δ 1.37(9H, s), 3.09(2H, m), 3.72(3H, s),  
4.38-4.80(8H, s), 6.85(1H, t), 7.84(1H, t).

Synthesis of (22b)

25 The compound (22b) was prepared by the same method as for  
the compound (22a) using  
N-tert-butoxycarbonylpropylenediamine instead of  
N-tert-butoxycarbonylethylenediamine.

22b:

<sup>1</sup>HNMR (DMSO-d<sub>6</sub>) δ 1.38 (9H, s) , 1.57-1.63 (2H, m, J=6.8, 7.1 Hz) , 2.97-3.01 (2H, q, J=6.3, 6.6 Hz) , 3.14-3.18 (2H, q, J=6.3 Hz) , 3.72 (3H, s) , 4.37-4.44 (4H, m) , 4.71-4.81 (4H, m) , 6.81-6.83 (1H, t, J=5.6 Hz) , 7.77-7.80 (1H, t, J=5.6 Hz) ,

Synthesis Example 11 of the material compounds: a general synthesis method of ferrocene derivatives (23a), (23b) and  
10 (23c)

The compound (22a) (10.5 g, 20 mmol) was dissolved in methanol (200 mL), mixed with aqueous solution of sodium hydroxide (0.5 M) (200 mL, 40 mmol) and stirred for 12 hours  
15 at 80°C. After being cooled to a room temperature, the solution was adjusted to pH 3.0 with citric acid and washed with water. The resulting organic layer was collected and dehydrated with anhydrous sodium sulfate, and the solvent was distilled out under a reduced pressure. The residue  
20 was dissolved in methylene chloride (220 ml), and mixed with ethyl ester hydrochloride of β-alanine (3.5 g, 22.8 mmol), triethylamine (10.8 ml, 77.5 mmol) and 2-chloro-1-methyliodide (6.7 g, 27.4 mmol) under argon atmosphere. After being stirred for two hours at a room  
25 temperature, the solution was washed with water. The resulting organic layer was collected and dehydrated with anhydrous sodium sulfate. The solvent was distilled out under a reduced pressure, and the residue was purified with

silica gel chromatography to give the compound (23a) (8.7 g, 85 %).

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.18-1.21 (3H, t, J = 7.1 Hz) 1.32 (9H, s),  
 5 2.59-2.61 (2H, t, J = 6.3 Hz), 3.32 (2H, m), 3.42-3.45 (2H, dd, J = 5.4 Hz), 3.57-3.60 (2H, dd, J = 6.3 Hz), 4.07-4.12 (2H, dd, J = 7.1 Hz), 4.27 (3H, t, J = 2.0 Hz), 4.29-4.30 (3H, t, J = 1.8 Hz) 4.46 (4H, d, J = 1.5 Hz), 5.89 (1H, m), 7.34-7.37 (1H, t, J = 1.8 Hz), 7.55-7.57 (1H, t, J = 1.8 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 14.0, 28.2, 33.9, 35.3, 40.2, 40.5, 60.4, 70.4, 70.5, 70.9, 71.0, 77.6, 78.0, 78.9, 156.5, 170.4, 10 170.5, 172.2 MS m/z calcd for C<sub>23</sub>H<sub>34</sub>FeN<sub>3</sub>O<sub>6</sub><sup>+</sup> : 516.17970, found 516.23203.

15 The compounds (23b) and (23c) were obtained by reacting the compounds (22a) and (22b), respectively, with an appropriate ester of an amino acid in accordance with the above general synthesis method.

20 23b:

<sup>1</sup>H NMR (500 MHz, DMSO) δ 1.16-1.19 (3H, t, j=7.1 Hz), 1.38 (9H, s), 1.75-1.78 (2H, m, j=7.1 Hz), 2.35-2.38 (2H, m), 3.09-3.11 (2H, m, j=5.9, 6.1 Hz), 3.18-3.23 (2H, q, j=5.9, 6.1, 6.6 Hz), 4.03-4.08 (2H, q, j=7.1 Hz), 4.29-4.31 (4H, m), 4.69-4.72 (4H, m), 6.90 (1H, t), 7.86-7.88 (1H, t, j=5.6 Hz), 7.90-7.92 (1H, t, j=5.6 Hz)

23c:

<sup>1</sup>H NMR (500 MHz, DMSO) δ 1.18-1.21 (3H, t, j=7.1 Hz) , 1.38 (9H, s) , 1.59-1.62 (2H, m, j=7.1 Hz) , 2.56-2.59 (2H, t, j=6.8 Hz), 2.97-3.01 (2H, q, j=6.6 Hz) , 3.15-3.19 (2H, q, j=6.6, 6.8 Hz) , 3.39-3.43 (2H, q, j=6.6, 7.1 Hz) , 5 4.07-4.11 (2H q, j=7.1 Hz) , 4.28-4.30 (4H, m,) , 4.69-4.70 (4H, m) , 6.81-6.83 (1H, t) , 7.82-7.85 (1H, t, j=5.9 Hz) , 7.96-7.98 (1H, t, j=5.8 Hz)

Synthesis Example 12 of the material compounds: a general  
10 synthesis method of ferrocene derivatives (24a), (24b) and  
(24c)

The compound (23a) (2.0 g, 3.38 mmol) was dissolved in methanol (39 mL), mixed with aqueous solution of sodium 15 hydroxide (0.1 M) (41 mL, 4.07 mmol) and stirred for 12 hours at a room temperature. The solution was adjusted to pH 3.0 with 1 N hydrochloric acid and washed with water. The resulting organic layer was collected and dehydrated with anhydrous sodium sulfate, so that the solvent was 20 distilled out under a reduced pressure. The residue was purified with silica gel chromatography to give the compound (24a) (1.84 g, 97 %).

<sup>1</sup>HNMR (DMSO-d<sub>6</sub>) δ 1.37 (9H, s) , 2.49-2.53(2H, m), 25 3.07-3.10(2H, dd, J = 6.1 Hz) , 3.19-3.22 (2H, dd, J = 6.1 Hz) , 3.36-3.40 (2H, dd, J = 6.3 Hz) , 4.28-4.29(4H, t, J = 1.7 Hz) , 4.69-4.71(4H, m) , 6.91-6.94(1H, t, J = 5.6 Hz) , 7.83-7.86(1H, t, J = 5.7 Hz) , 7.93-7.95(1H, t, J

= 5.4 Hz ), 12.25 (1H, s );  $^{13}\text{CNMR}$  (DMSO-d<sub>6</sub>)  $\delta$  28.5, 34.3, 35.5, 38.8, 69.8, 71.7, 71.8, 72.2, 77.8, 77.8, 77.9, 79.4, 155.9, 168.7, 168.8, 173.3. MS m/z calcd for C<sub>22</sub>H<sub>30</sub>FeN<sub>3</sub>O<sub>6</sub><sup>+</sup>: 488.14840 , found 488.22584.

5

The compounds (24b) and (24c) were obtained by using the compounds (23b) and (23c), respectively, as a starting material in accordance with the above general synthesis method.

10

24b:

$^1\text{H NMR}$  (500 MHz, DMSO)  $\delta$  1.38 (9H, s ), 1.72-1.77 (2H, m, J=7.1, 7.3 Hz), 2.28-2.31 (2H, t, J=7.3 Hz), 3.08-3.11 (2H, q, J=6.3 Hz), 3.18-3.24 (4H, m, J=6.3 Hz), 4.29-4.30 (4H, s ), 4.69-4.72 (4H, m), 6.91-6.93 (1H, t, J=5.6 Hz), 7.87-7.92 (2H, m, J=5.6, 5.8 Hz), 12.1 (1H, s ).

24c:

$^1\text{H NMR}$  (500 MHz, DMSO)  $\delta$  1.38 (9H, s ), 1.59-1.63 (2H, m, J=6.8 Hz), 2.97-3.01 (2H, m, J=6.6 Hz), 3.15-3.19 (2H, q, J=6.6, 6.8 Hz), 3.36-3.40 (2H, m, J=6.8 Hz) 4.28-4.29 (4H, t), 4.70-4.72 (4H, m), 6.81-6.83 (1H, t, J=5.6 Hz), 7.81-7.83 (1H, t, J=5.9 Hz), 7.92-7.94 (1H, t, J=5.4 Hz), 12.2 (1H, s ).

25

Synthesis Example 13 of the material compounds: a general synthesis method of ferrocene derivatives (25a), (25b) and (25c)

The compound 15 (1.64 g, 3.28 mmol) was dissolved in methanol (13.1 mL), mixed with 10 % Pd/C (164 mg) and stirred under hydrogen atmosphere for four hours. 10% Pd/C was removed by filtration with cerite, and the solvent was distilled out under a reduced pressure. The residue was dissolved in methylene chloride (32.8 ml), mixed with the compound (24a) (1.60 g, 3.28 mmol), DCC (744 mg, 3.61 mmol) and hydroxybenzotriazole (488 mg, 3.61 mmol), and stirred for one hour at a room temperature. The precipitated white powder was filtered out and washed with water. An organic layer was collected and dehydrated with anhydrous sodium sulfate, so that the solvent was distilled out under a reduced pressure. The residue was purified with silica gel chromatography to give the compound (25a) (2.52 g, 82 %).

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 1.37 (9H, s), 1.70-1.73 (2H, t, J = 7.1 Hz), 2.42 (6H, s), 2.55-2.61 (4H, m), 3.09-3.47 (16H, m), 3.80 (3H, s), 3.83 (3H, s), 3.95 (3H, s), 4.28-4.29 (4H, m), 4.71-4.73 (4H, m), 6.92 (1H, d, J = 1.5 Hz), 6.94 (1H, d, J = 1.7 Hz), 7.21 (1H, d, J = 1.7 Hz), 7.29 (1H, d, J = 1.5 Hz), 7.51 (1H, s), 7.88-7.91 (1H, t, J = 5.5 Hz), 8.00-8.01 (1H, t, J = 5.5 Hz), 8.14-8.16 (1H, t, J = 5.4 Hz), 8.31 (1H, s), 9.95 (1H, s), 10.23 (1H, s);  
<sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ 26.1, 28.4, 35.0, 36.0, 36.0, 36.2, 36.4, 36.6, 43.9, 56.2, 69.8, 71.7, 77.8, 77.9, 79.3, 104.3, 104.8, 114.9, 118.1, 119.3, 121.4, 122.0, 122.3, 123.3, 134.3,

136.2, 155.9, 158.8, 161.4, 168.1, 168.7, 168.8. MS m/z  
 calcd for  $C_{44}H_{59}FeN_{12}O_8^+$  : 939.39282 , found 939.2523.

The compounds (25b) and (25c) were obtained by using the  
 5 compounds (24b) and (24c), respectively, as a starting  
 material in accordance with the above general synthesis  
 method.

25b:

10  $^1H$  NMR (500 MHz, DMSO)  $\delta$  1.38 (9H, s) , 1.59-1.63 (2H, m,  
 j=7.1 Hz) , 1.81-1.84 (2H, m, j=7.3 Hz) , 2.14 (6H, s) ,  
 2.23-2.26 (2H, t, j=7.1 Hz) , 2.31-2.34 (2H, m, j=7.3 Hz)  
 , 3.09-3.12 (2H, m, j=5.9 Hz) , 3.17-3.25 (6H, m) , 3.80  
 (3H, s) , 3.84 (3H, s) , 3.97 (3H, s) , 4.32 (4H, s) , 4.70-4.73  
 15 (4H, m) , 6.89-6.93 (3H, m, j=1.7, 1.9 Hz) , 7.22 (1H, d,  
 j=1.7 Hz) , 7.28 (1H, d, j=1.7 Hz) , 7.53 (1H, s) , 7.89-7.94  
 (2H, m, j=5.6, 5.9 Hz) , 8.09-8.11 (1H, t, j=5.6 Hz) 9.88  
 (1H, s) , 9.95 (1H, s) , 10.22 (1H, s)

20 25c:

$^1H$  NMR (500 MHz, DMSO)  $\delta$  1.38 (9H, s) , 1.58-1.64 (4H, m,  
 j=6.8, 7.1 Hz) , 2.14 (6H, s) , 2.23-2.26 (2H, t, j=7.1  
 Hz) , 2.56-2.58 (2H, t, j=7.1 Hz) , 2.98-3.02 (2H, m, j=6.3,  
 6.6 Hz) , 3.16-3.21 (4H, m, j=6.3, 6.6 Hz) , 3.43-3.47 (2H,  
 25 m, j=6.6, 6.8 Hz) , 3.80 (3H, s) , 3.84 (3H, s) , 3.97 (3H,  
 s) , 4.28-4.29 (4H, m) , 4.71-4.73 (4H, m) 6.81-6.83 (1H,  
 t, j=5.6 Hz) , 6.89 (1H, d, j=2.0 Hz) , 6.93 (1H, d, j=2.0  
 Hz) , 7.22 (1H, d, j=1.7 Hz) , 7.30 (1H, d, j=1.7 Hz) ,

7.52 (1H, s) 7.83-7.85 (1H, t,  $j=5.9$  Hz) , 7.96-7.97 (1H, t,  $j=5.6$  Hz) , 8.08-8.11 (1H, t,  $j=5.6$  Hz) , 9.95 (1H, s) , 9.97 (1H, s), 10.23 (1H, s).

5 Synthesis Example 14 of the material compounds: a general synthesis method of ferrocene derivatives (26a), (26b) and (26c)

The compound (25a) (1.5 g, 1.6 mmol) was dissolved in 10%  
10 trifluoroacetic acid/methylene chloride (25.3 ml, 32 mmol)  
and stirred for 5 hours at a room temperature. The solution  
was washed with water. An organic layer was collected and  
dehydrated with anhydrous sodium sulfate, so that the  
solvent was distilled out under a reduced pressure. The  
15 residue was purified with silica gel chromatography to give  
the compound (26a) (1.04 g, 78 %).

$^1\text{H}$ NMR ( $\text{CDCl}_3$ )  $\delta$  1.66-1.71 (2H, m), 2.20 (6H, s), 2.35-2.38 (2H,  
t,  $J = \text{Hz}$ ), 2.74 (2H, s), 2.97 (2H, m), 3.39-3.40 (2H, m),  
20 3.47 (2H, m), 3.69 (2H, s), 3.84 (3H, s), 3.87 (3H, s), 3.97 (3H,  
s), 4.27 (2H, s), 4.31 (2H, s), 4.58-4.60 (4H, d,  $J=$ ), 6.70  
(1H, s), 6.90 (1H, s), 7.20 (1H, s), 7.23 (1H, s), 7.38 (1H, s),  
7.45-7.46 (1H, m), 7.76 (2H, m), 7.83 (1H, m), 9.07 (1H, br),  
25 9.21 (1H, br), 9.88 (1H, s); MS m/z calcd for  $\text{C}_{39}\text{H}_{51}\text{FeN}_{12}\text{O}_6^+$   
: 839.34039 , found 839.33831.

The compounds (26b) and (26c) were obtained by using the  
compounds (25b) and (25c), respectively, as a starting

material in accordance with the above general synthesis method.

26b:

5     <sup>1</sup>H NMR (500 MHz, DMSO) δ 1.58-1.64 (2H, m, j=7.1 Hz) ,  
       1.80-1.85 (2H, m) , 2.14 (6H, s) , 2.23-2.27 (2H, t, j=7.1  
       Hz) , 2.31-2.36 (2H, m, j=7.1 Hz) , 2.69-2.72 (2H, t, j=6.6  
       Hz) , 3.17-3.23 (6H, m) , 3.81 (3H, s) , 3.84 (3H, s) , 3.97  
       (3H, s) , 4.29-4.33 (4H, m) , 4.70-4.73 (4H, m) 6.88 (1H,  
 10    s) , 6.93 (1H, s) , 7.22 (1H, s) , 7.29 (1H, m) , 7.53 (1H,  
       s) , 7.86-7.88 (1H, t, j=5.6 Hz) , 7.92-7.97 (2H, m) ,  
       8.10-8.12 (1H, t, j=5.6 Hz) , 9.90-9.91 (1H, d) , 9.97 (1H,  
       s) .

15    26c:

1     <sup>1</sup>H NMR (500 MHz, DMSO) δ 1.59-1.62 (4H, m) , 2.14 (6H, s)  
       , 2.23-2.26 (2H, t, j=7.1 Hz) , 2.57-2.59 (2H, t) , 3.19-3.20  
       (2H, m) , 3.24-3.25 (2H, m) , 3.45-3.47 (2H, m) , 3.80 (3H,  
       s) , 3.84 (3H, s) , 3.97 (3H, s) , 4.29 (4H, s) , 4.71 (4H,  
 20    d) 6.89 (1H, d, j=1.5 Hz) , 6.94 (1H, s) , 7.22 (1H, s)  
       , 7.30 (1H, s) , 7.53 (1H, s) , 7.93-7.95 (1H, t, j=5.4  
       Hz) 7.99-8.10 (2H, m, j=5.1 Hz) , 9.99-10.02 (1H, m, j=5.1  
       Hz) .

25    Synthesis Example 15 of the material compound: Polyamide  
       (28)

The compound 15 (806 mg, 1.61 mmol) was dissolved in methanol

(6.5 mL), mixed with 10 % Pd/C (80.5 mg) and stirred under hydrogen atmosphere for four hours. 10% Pd/C was removed by filtration with cerite, and the solvent was distilled out under a reduced pressure. The residue was dissolved in methylene chloride (16 ml), mixed with 2-N-Boc-4-N-Fmoc-2,4-diaminobutylic acid (710 mg, 1.61 mmol), DCC (332.2 mg, 1.61 mmol) and hydroxybenzotriazole (217.5 mg, 1.61 mmol), and stirred for one hour at a room temperature. The precipitated white powder was filtered out, mixed with 5% piperidine/dimethylformamide (27.7 ml, 16.1 mol), stirred for 30 min at a room temperature and washed with water. An organic layer was collected and dehydrated with anhydrous sodium sulfate, so that the solvent was distilled out under a reduced pressure. The residue was purified with silica gel chromatography to give the compound (28) (453 mg, 42 %).

<sup>1</sup>HNMR (CDCl<sub>3</sub>) δ 1.44 (9H, s), 1.71-1.76 (2H, t, J = 6.3Hz), 1.91-1.98 (2H, t, J = 6.3Hz), 2.26 (6H, s), 2.41-2.44 (2H, t, J = 6.3Hz), 2.90-2.95 (2H, m), 3.44-3.47 (2H, dd, J = 5.7 Hz), 3.83 (3H, s), 3.91 (3H, s), 4.01 (3H, s), 4.52 (1H, s), 6.46 (1H, s), 6.63 (1H, s), 6.67 (1H, s), 7.15 (1H, s), 7.26 (1H, s), 7.37 (1H, s), 7.77 (1H, s), 8.24 (1H, s), 9.10 (1H, s), 9.64 (1H, s). MS m/z calcd for C<sub>31</sub>H<sub>48</sub>N<sub>11</sub>O<sub>6</sub> <sup>+</sup> : 670.37890, found 670.33883.

Synthesis Example 16 of the material compound: Polyamide (29)

The compound (10) (303.6 mg, 0.772 mmol) dissolved in methylene chloride (7.0 ml) was mixed with diisopropylethylamine (0.239 ml, 1.404 mmol) and 5 O-(benzotriazole-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (532.5 mg, 1.404 mmol), and stirred for 30 min at a room temperature. The compound (19) (470 mg, 0.702 mmol) dissolved in methylene chloride (7.0 ml) was added to the resulting solution and stirred for one 10 hour. The resulting mixture was washed with water. An organic layer was collected and dehydrated with anhydrous sodium sulfate, so that the solvent was distilled out under a reduced pressure. The residue was re-precipitated from chloroform and diethylether to give the compound (29) (673 15 mg, 94 %).

<sup>1</sup>HNMR (DMSO-d<sub>6</sub>) δ 1.34 (9H, s),  
1.70-1.73 (2H, t,) 1.75-193 (2H, m), 2.41 (6H, s), 2.61 (2H.m),  
3.19-3.22 (2H, dd, J = 1.7 Hz), 3.27-3.37 (2H, m), 3.80 (3H, s),  
20 3.83 (3H, s), 3.85 (3H, s), 3.92 (3H, s), 3.96 (3H, s),  
3.98 (3H, s), 4.08-4.13 (1H, m) 6.94 (1H, d, J = 1.5Hz), 6.97 (1H,  
s), 7.02 (1H, s), 7.16 (1H, d, J = 1.5 Hz), 7.22 (1H, s),  
7.26 (1H, d, J = 1.2 Hz), 7.37 (1H, s), 7.38 (1H, d, J = 1.5 Hz),  
7.49 (1H, s), 7.53 (1H, s), 7.97-7.80 (1H,,t, J = 5.5 Hz),  
25 8.15-8.17 (1H, t, J = 5.5 Hz), 9.94 (1H, s), 9.99 (1H, s),  
10.27 (1H, s), 10.34 (1H, s), 10.36 (1H, s); <sup>13</sup>CNMR (DMSO-d<sub>6</sub>) δ  
26.1, 28.4, 32.2, 35.1, 35.3, 35.7, 36.3, 36.4, 36.5, 36.6,  
38.4, 43.9, 52.6, 56.1, 78.4, 79.4, 104.4, 105.0, 106.1,

114.5, 115.0, 118.2, 119.5, 119.7, 121.4, 121.7, 121.9,  
122.1, 122.4, 123.3, 126.6, 127.2, 134.2, 134.4, 136.2,  
136.2, 138.9, 155.6, 155.9, 156.3, 158.9, 158.9, 161.4,  
169.4. MS m/z calcd for C<sub>47</sub>H<sub>63</sub>N<sub>18</sub>O<sub>9</sub><sup>+</sup>: 1023.50254 , found  
5 1023.49821.

Example 3: General synthesis method of the compounds (1a),  
(1b), (1c) and (2)

10 The compound (26a) (187.6 mg, 0.477 mmol) dissolved in  
methylene chloride (4.77 ml) was mixed with  
diisopropylethylamine (0.162 ml, 0.947 mmol) and  
O-(benzotriazole-yl)-N,N,N',N'-tetramethyluronium  
hexafluorophosphate (361.8 mg, 0.954 mmol), and stirred  
15 for 30 min at a room temperature. The compound (10) (400  
mg, 0.477 mmol) dissolved in methylene chloride (4.8 ml)  
was added to the resulting solution and stirred for one  
hour. The resulting mixture was washed with water. An  
organic layer was collected and dehydrated with anhydrous  
20 sodium sulfate, so that the solvent was distilled out under  
a reduced pressure. The residue was purified with the  
silica gel chromatography to give the compound (1a) (349  
mg, 61 %).

25 <sup>1</sup>HNMR(DMSO-d<sub>6</sub>) δ 1.59-1.65( 2H, m ), 2.15( 6H, s ),  
2.26-2.28( 2H, t, J = 7.1 Hz ), 2.56-2.59( 2H, t, J = 7.0  
Hz ), 3.18-3.22( 2H, m, ), 3.37-3.50( 6H, m ), 3.81( 3H, s  
, 3.83( 3H, s ), 3.85( 3H, s ), 3.94( 3H, s ), 3.97( 3H,

s), 3.99 (3H, s), 4.27-4.30 (4H, m), 4.73 (4H, s), 6.88 (1H, d, J = 1.7 Hz), 6.94 (1H, d, J = 1.5 Hz), 7.02 (1H, s), 7.16 (1H, d, J = 1.7 Hz), 7.19 (1H, d, J = 1.5 Hz), 7.26 (1H, d, J = 1.7 Hz), 7.35-7.36 (2H, m), 7.46 (1H, s), 7.49 (1H, s), 7.86-7.87 (1H, t, J = 5.6 Hz), 7.91-7.93 (1H, t, J = 5.5 Hz), 7.98-7.80 (1H, t, J = 5.5 Hz), 8.05-8.08 (1H, t, J = 5.7 Hz), 9.77 (1H, s), 9.88 (1H, s), 10.03 (1H, s), 10.06 (1H, s), 10.19 (1H, s);  $^{13}\text{C}$ NMR (DMSO-d<sub>6</sub>)  $\delta$  27.2, 35.1, 35.1, 35.3, 36.0, 36.1, 36.2, 36.4, 36.5, 37.4, 38.8, 39.0, 45.3, 57.3, 69.8, 71.7, 71.8, 77.9, 78.0, 79.3, 104.1, 104.8, 106.1, 114.6, 114.9, 118.0, 119.4, 119.6, 121.4, 1221.7, 122.2, 122.3, 122.4, 123.5, 126.6, 127.2, 134.1, 134.4, 136.2, 138.9, 155.9, 156.3, 158.9, 158.9, 159.2, 161.2, 168.2, 168.6, 169.0. MS m/z calcd for C<sub>55</sub>H<sub>66</sub>FeN<sub>19</sub>O<sub>9</sub><sup>+</sup>

15 : 1192.46403, found 1192.37089.

The compounds (1b) and (1c) were obtained by using the compounds (26b) and (26c), respectively, instead of the compound (26a) as a starting material in accordance with 20 the above general synthesis method. The compound (2) was obtained by using the compounds (26a) and (20) as a starting material in accordance with the above general synthesis method.

25 1b :

$^1\text{H}$  NMR (500 MHz, DMSO)  $\delta$  1.59-1.63 (2H, m), 1.80-1.83 (2H, m), 2.14 (6H, s), 2.23-2.26 (2H, t, j=6.8 Hz), 2.31-2.34 (2H, t, j=7.2 Hz), 3.18-3.23 (4H, m), 3.44-3.50 (4H, m)

, 3.80 (3H, s) , 3.83 (3H, s) , 3.85 (3H, s) , 3.94 (3H, s) , 3.96 (3H, s) , 3.99 (3H, s) , 4.29-4.32 (4H, d) , 4.74 (4H, s) 6.90 (1H, s) , 6.93 (1H, s) , 7.04 (1H, s) , 7.16 (1H, s) , 7.22 (1H, s) , 7.28 (1H, s) 7.39 (1H, m) , 7.50 (1H, s) , 7.53 (1H, s) , 7.91-7.93 (1H, t, j=5.0 Hz) , 8.04 (1H, t) , 8.11-8.12 (1H, t, j=5.1 Hz) , 8.14-8.16 (1H, t, j=5.3 Hz) , 9.89 (1H, s) , 9.98 (1H, s) , 10.23 (1H, s) , 10.24 (1H, s) , 10.38 (1H, s).

10 1c:

<sup>1</sup>H NMR (500 MHz, DMSO) δ 1.58-1.63 (2H, m, j=7.1 Hz) , 1.72-1.75 (2H, m, j=6.7 Hz) , 2.13 (6H, s) , 2.22-2.25 (2H, t, j=7.1 Hz) , 2.56-2.58 (2H, t, j=7.0 Hz) , 3.17-3.21 (2H, q, j=6.1, 6.6 Hz) , 3.22-3.26 (2H, q, j=6.1, 6.6 Hz) , 3.44-3.48 (2H, q, j=6.1, 6.8 Hz) , 3.80 (3H, s) , 3.83 (3H, s) , 3.85 (3H, s) , 3.94 (3H, s) , 3.96 (3H, s) , 3.99 (3H, s) , 4.30-4.32 (4H, m) , 4.72-4.75 (4H, m) , 6.89 (1H, d, j=2.0 Hz) , 6.92 (1H, d, j=1.7 Hz) , 7.03 (1H, d, j=1.7 Hz) , 7.17 (1H, d, j=1.7 Hz) , 7.21 (1H, d, j=1.7 Hz) , 7.30 (1H, d, j=1.7 Hz) 7.39 (2H, d, j=2.2 Hz) , 7.50 (1H, s) , 7.52 (1H, s) , 7.93-7.95 (1H, t, j=5.9 Hz) , 7.99-8.01 (1H, t, j=5.9 Hz) , 8.14-8.16 (1H, t, j=5.9 Hz) , 9.97 (1H, s) , 9.99 (1H, s) , 10.24 (1H, s) , 10.27 (1H, s) , 10.38 (1H, s).

25

Compound (2):

<sup>1</sup>HNMR(DMSO-d<sub>6</sub>) δ 1.59-1.62(2H, t, J=7.0Hz), 2.14(6H, s), 2.23-2.25(2H, t, J=7.0Hz), 2.55-2.60(4H, m), 3.17-3.21(2H,

m), 3.35-3.37(2H, m), 3.42-3.50(6H, m), 3.80(3H.s),  
3.83(3H,s), 3.91(3H, s), 3.93(3H, s), 3.96(3H, s),  
4.25-4.26(2H, m), 4.29(2H, m), 4.72-4.73(2H, m),  
4.74-4.75(2H, m), 6.89(1H, d, J=1.8Hz), 6.93(1H, d,  
5 J=1.7Hz), 6.95(1H, s), 7.21(1H, d, J=1.7Hz), 7.31(1H, d,  
J=1.6Hz) 7.32(1H, s) 7.41(1H, s) 7.52(1H, s)  
7.99-8.01(2H m) 8.10-8.12(2H, m) 8.34(1H, t, J=6.0Hz)  
9.95(1H, s) 10.0(1H s) 10.2(1H, s) 10.2(1H, s)  
10.3(1H, s).

10

Example 4: Synthesis of Fc-PIA 3 (Compound 3)

The compound (29) (300 mg, 0.293 mmol) was dissolved in  
20% trifluoroacetic acid/methylene chloride (1.16 ml, 2.93  
15 mmol) and stirred for one hour at a room temperature. The  
solution was washed with water. An organic layer was  
collected and dehydrated with anhydrous sodium sulfate,  
so that the solvent was distilled out under a reduced  
pressure. The residue was dissolved in methylene chloride  
20 (2.9 ml), mixed with diisopropylamine (0.10 ml, 0.586 mmol),  
ferrocene carboxylic acid (67.4 mg, 0.293 mmol) and  
O-(benzotriazole-yl)-N,N,N',N'-tetramethyluronium  
hexafluorophosphate (111.1 mg, 0.293 mmol), and stirred  
for 10 hours at a room temperature. The resulting mixture  
25 was washed with water. An organic layer was collected and  
dehydrated with anhydrous sodium sulfate, so that the  
solvent was distilled out under a reduced pressure. The  
residue was purified with silica gel chromatography to give

the compound (3) (204 mg, 61 %).

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 1.57-1.62 (2H, m), 2.12 (6H, s),  
 2.22-2.24 (2H, t, J = 7.0 Hz), 3.16-3.20 (2H, m),  
 5 3.37-3.43 (2H, m), 3.80 (3H, s), 3.83 (3H, s), 3.85 (3H, s),  
 3.93 (3H, s), 3.96 (3H, s), 3.98 (3H, s), 4.22 (5H, s),  
 4.36-4.37 (2H, m), 4.85 (2H, m), 4.94 (2H, m), 6.86 (1H, d, J =  
 1.7 Hz), 6.99 (1H, d, J = 1.7 Hz), 7.02 (1H, d, J = 1.0 Hz),  
 7.17 (1H, d, J = 1.7 Hz), 7.20 (1H, d, J = 1.7 Hz), 7.28 (1H, d,  
 10 J = 1.7 Hz), 7.37 (1H, s), 7.38 (1H, d, J = 1.7 Hz), 7.49 (1H, s),  
 7.52 (1H, s), 7.86-7.87 (1H, d, J = 1.7 Hz), 8.05-8.10 (2H, m),  
 9.95 (1H, s), 10.04 (1H, s), 10.24 (1H, s), 10.25 (1H, s),  
 10.27 (1H, s), 10.36 (1H, s); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ 27.2, 35.1,  
 35.1, 35.3, 36.0, 36.1, 36.2, 36.4, 36.5, 37.4, 38.8, 39.0,  
 15 45.3, 57.3, 69.8, 71.7, 71.8, 77.9, 78.0, 79.4, 104.1, 104.8,  
 106.1, 114.6, 114.9, 118.0, 119.4, 119.6, 121.4, 121.7,  
 122.0, 122.3, 122.4, 123.5, 126.6, 127.2, 134.1, 134.4,  
 136.2, 138.9, 155.9, 156.3, 158.9, 158.9, 159.2, 161.2,  
 168.2, 168.6, 169.0. MS m/z calcd for C<sub>53</sub>H<sub>63</sub>FeN<sub>18</sub>O<sub>8</sub><sup>+</sup> :  
 20 1135.44257, found 1135.43889.

Example 5: Properties of the compounds 1a, 2 and 3 as ligand

Similarly with Example 2, the binding of the above compounds  
 25 to the target DNA (5' GACTGCGTAGG3' / 3' CTGACGCATCC5') was  
 detected with CD spectrum in order to confirm its utility  
 as a ligand for the double-stranded helical DNA. The  
 results are in Fig.2. It is well known that elliptical

polarization at 300 - 360 nm was induced by the interaction with a minor groove (Pilch, D.S.; Poklar, N.; Baird, E.E.; Dervan, P.B.; Breslauer, K.J. Biochemistry, 2002, 38, 2143-2151). As a result, the addition of the solution of 5 target DNA (5.0  $\mu$ M) caused concentration-dependent increase of a positive cotton effect.

Example 6: Detection of the electrochemical properties by cyclic voltammetry (CV)

10

The results of the experiment about the electrochemical properties of the present compounds (1a) and (3) with a cyclic voltammetry (CV) are shown in Fig. 3. They indicate that the compounds according to the present invention have 15 the activities for the electrochemical reaction.

INDUSTRIAL APPLICABILITY

The preset invention may be utilized in various kinds of 20 industrial fields such as, for example, development of drugs, clinical inspection (test), screening of pharmaceuticals, safety test of compounds, food evaluation, medical inspection, medicolegal inspection, brewing, agriculture, forestry, fishery, and livestock farming.

25